EFFECTS OF CO-INOCULATION OF ARBUSCULAR MYCORRHIZAL FUNGI AND *RHIZOBIUM* ON THE TRIPARTITE ASSOCIATION WITH FIELD PEA (*Pisum sativum*) AND LENTIL (*Lens culinaris*) UNDER SASKATCHEWAN FIELD CONDITIONS

A Thesis Submitted to the College of Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

in the Department of Soil Science

University of Saskatchewan

Saskatoon

By

Anisha Biswaray

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department of Soil Science or the Dean of the College of Agriculture and Bioresources. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use that may be made of any material in my thesis. Requests for permission to copy or to make other uses of materials in this thesis, in whole or part, should be addressed to:

Head, Department of Soil Science
University of Saskatchewan
Saskatoon, Saskatchewan
Canada, S7N 5A8

DISCLAIMER

Any reference to specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, in this thesis does not constitute or imply its endorsement, recommendation, or favouring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan, and shall not be used for advertising or product endorsement purposes.

ABSTRACT

Arbuscular mycorrhizal fungi were recently introduced in western Canada as a commercial biofertilizer for field crop production. Mycorrhizal inoculant use is an established practice in land reclamation and horticultural activities; however, it is only an emerging interest in agricultural systems. Key questions need to be answered regarding its impact on growth, nutrient uptake and yield responses in field crops, particularly for legumes, when co-inoculated with nitrogen (N) fixing rhizobial inoculants. Field experiments were conducted at various locations in Saskatchewan in 2012 and 2013 to investigate the response of pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) to the application of a commercial AMF inoculant MYKE® PRO GR (*Glomus intraradices*) (Premier Tech Ltd.) at the recommended application rate and twice the recommended rate (7.5 kg ha^{-1} and 15 kg ha^{-1} respectively) when applied with and without *Rhizobium* inoculant (Nodulator®, Becker Underwood). All the treatments were repeated with and without phosphorus (16.8 kg ha^{-1}). The results suggest that application of P fertilizer in combination with AMF and *Rhizobium* significantly enhanced mid-season biomass, and N and P accumulation, seed nutrient yield and biological N fixation. Seed yield of lentil and pea were unaffected by AMF inoculation. It was concluded that the tripartite symbiotic association can have synergistic responses in legumes under prairie field conditions, but the responses can depend on host species and other biotic and abiotic parameters.

ACKNOWLEDGMENTS

I would like to thank my supervisors, Drs. Fran Walley and Jim Germida for being a source of inspiration and motivation these past years. I am indebted to them for having faith in me and for constantly supporting me through my program. They have provided me with a rich experience in research and life. My deepest appreciation to my "Gurus" for every bit of knowledge and experience I gained, without their guidance and persistent help this thesis would not have been possible.

I would like to thank my committee Chair Dr. Angela Bedard Haughn and committee member Dr. Chantal Hamel for their continuous and valuable support and suggestions throughout the program. I sincerely appreciate their patience over the last couple of years to see this research through to the end. I would also like to thank Dr. Steven Shirtliff for serving as the external examiner, and for valuable suggestions.

I sincerely express my gratitude to the Saskatchewan Pulse Growers and Natural Science and Engineering Research Council (NSERC) for financial support of the research project. I would also like to express my appreciation to College of Graduate Studies and Research for various scholarships granted during my program.

Special thanks to Dr. Mandantha Wijesinghe for her valuable time, inputs and help, Benjamin Flath for his help in field activities and field co-operators at Westwind Ag research, CSIDC and AAFC. I would also like to say thanks to all the wonderful lab mates of 5E25 and 5C19, their wonderful sense of humour and camaraderie will be missed very much. Also, I want to thank other graduate students and faculty of Department of Soil Science as well, for their good will and wishes. I could not have finished without their help and support. Last, and definitely not the least, my parents and grandparents for their continued blessings and encouragement, my siblings Manisha, Ishan and cousins Anurag, Soma for being the best cheerleaders, and Abhijeet for his love and patience as well as all my friends across India and Canada.

DEDICATION

This thesis is dedicated to my grandpa “Ajja” who has always been my source of inspiration. His boundless energy and constant encourgament has always spurred me to achieve my goals. His unconditional love and support has been my pillar of strength through thick and thin.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AMF	Arbuscular mycorrhizal fungi
AP	Appresorium
CEC	Cation exchange capacity
CSP	Common symbiotic pathway
LCO	Lipochitooligosaccharide
LysM	Lysin-motif
MPN	Most probable number
Ndfa	Nitrogen directly fixed from atmosphere
NH_4^+	Ammonium ion
NO_3^-	Nitrate
OTU	Operational taxonomic unit
PAM	Peri-arbuscular membrane
PCR	Polymerase chain reaction
PGPR	Plant growth promoting rhizobacteria
P_i	Orthophosphate
P_iT	Orthophosphate transporters
PPA	Pre-penetration apparatus
RL	*Rhizobium*-legume

1. INTRODUCTION

Microorganisms are an important part of the biotic component of soil, and they are either directly or indirectly responsible for vital soil processes, including the biogeochemical cycling of nutrients, hydrological processes, and soil quality maintenance. Microbial activities mediate various soil processes and determine nutrient availability or limitation to plants, both of which significantly influence on plant health and productivity (Shaw and Burns, 2006; Sardans et al., 2008). Therefore, microorganisms may sometimes act as indicators of soil health (Zhang and Xu, 2008). Complex interactions between soil, host plants, and symbiotic microorganisms that colonize plants further contribute to the overall health of the ecosystem (Kennedy 1998; Sheng et al., 2012). In this context, using microbial inoculants (biofertilizers) in agro-ecosystems has offered an environmentally sustainable alternative to the application of chemical fertilizers, specifically in areas that are recovering from the overuse of mineral fertilizers or those that are inherently nutrient deficient (Duponnois et al., 2001; Sarmiento et al., 2006). An understanding of soil microbial functions, coupled with the optimization or manipulation of existing microbial interactions, in agricultural systems can aid the development of soil microbial technologies and enhance crop productivity (Bolton et al., 1992; Kiers et al., 2003; Tikhonovich and Provorov, 2007).

Several groups of beneficial rhizospheric microbes are involved in symbiotic interactions with plant roots. These interactions often involve an intricate set of biochemical signalling between the microbe and the host, and infective organs are sometimes developed, as seen in mycorrhizas and root nodules. Arbuscular mycorrhizal fungi (AMF) and rhizobia are arguably the two most important beneficial soil symbiotic organisms in agricultural systems associated with legumes. The interaction between *Rhizobium* species and legumes is well documented, and rhizobial inoculants are an extensively used biofertilizer in legume cropping systems. Rhizobial inoculants contribute to the production of 22 million tons of nitrogen (N) per year in agricultural soils (Brockwell et al., 1995; Herridge et al., 2008), but the mycorrhizal relationship has only recently become a significant biofertilizer research topic (Smith and Read, 2008). Arbuscular mycorrhizal fungi are considered essential to the improvement of plant nutrition and soil quality (Gosling et al., 2006), and these fungi and *Rhizobium* are known to synergistically act in legumes to enhance plant health in controlled environmental conditions (Xavier and Germida, 2002). The

use of the AMF-*Rhizobium* consortium and other microbial organisms is essential to the general development of sustainable farming crop procedures, while lessening the use of chemical fertilizers.

Arbuscular mycorrhizal fungi are major symbiotic partners for most terrestrial plants, and they provide the plant with a range of nutritional benefits and increased protection against plant pathogens and environmental stresses. Arbuscular mycorrhizal fungi are ubiquitous in natural ecosystems, and they significantly influence plant community structure. Their contribution to plant health is significant in the case of nodulating legumes in farming systems. Legumes that get colonized with both *Rhizobium* and AMF show improved growth, overall health, and higher yields than non-symbiotic plants.

The tripartite symbiosis between AMF, rhizobia, and legumes is a prominent symbiotic association in that it could potentially impact agronomic parameters for legume production. The physiological and biochemical basis for the agronomic benefits of legume crops are a direct consequence of the tripartite symbiotic association, which is a complex set of exchanges between three functional partners: the legume, the *Rhizobium*, and the AMF. In the tripartite symbiotic association, AMF improves phosphorus uptake for plants. The higher phosphorus (P) concentration in the plant benefits the N-fixers and the plant's nitrogenase function (Bethlenfalvay and Newton, 1991; Barea et al., 1992). This leads to increased N fixation, which then promotes root and mycorrhizal development (Bethlenfalvay and Newton, 1991; Barea et al., 1992). Understanding the complex interactions taking place in the rhizosphere is crucial to improving agricultural productivity and maximizing the ecological benefits provided by microbial interactions. Mycorrhizas and *Rhizobium* bacteria are known to interact (Azcon et al., 1991; Saxena et al., 1997; Xavier and Germida, 2002), and these interactions can be synergistic between two apparently compatible AMF and *Rhizobium* strains (Xavier and Germida, 2002). According to Xavier and Germida (2002) a successful tripartite association significantly increased the yield and N nutrition in pea (*Pisum sativum* L.) plants. Previous studies concluded that the ability to fix N_2 directly from the atmosphere is enhanced in legumes in the presence of AMF (Barea et al., 2002a.). Toro et al. (1998) showed that N fixation rates in mycorrhizal alfalfa plants inoculated with *Rhizobium meliloti* were higher than the corresponding rates in non-mycorrhizal plants. Arbuscular mycorrhizal fungi are known to positively affect N fixation by influencing the energy producing pathways through enhanced P uptake (Mortimer et al., 2008).

The hormonal effects produced due to the mycorrhization on the roots and nodules are also known to affect N_2 fixation in tripartite symbioses (Franzini et al., 2010). However, antagonistic interactions were found by Rydlova and colleagues (2011) between AMF and *Sinorhizobium* on flax (a non-legume plant) yield on spoil bank clay, supporting species-specific interactions.

The tripartite endosymbiosis in legumes is established by exchanging mutually recognizable diffusible signals produced by plant and microbial partners. These signals are microbial exudates that are chemically lipo-chitooligosaccharides (LCOs) (Maillet et al., 2011). They are known to activate the signalling pathway called the common symbiotic pathway (CSP), which controls both *Rhizobium*-legume (RL) and AMF symbioses (Banba et al., 2008; Chen et al., 2007, 2008, 2009; Kouchi et al., 2010). Recent work has established that an AMF, *Glomus intraradices*, produces LCOs that activate the CSP, leading to the induction of gene expression and root branching in *Medicago truncatula* (Gough and Cullimore, 2011). Observations by different researchers that work with legume mutants show that both RL and AMF symbioses might be related in a complex manner, because mutant plants impaired following the accomplishment of root nodulation endosymbiosis are also unable to associate with Glomeromycota (Lima et al., 2009). This observation suggested a relationship between the pathways of AMF and RL symbioses (Kistner et al., 2005), giving rise to the idea of 'common symbiosis genes' (Kistner et al., 2005) and a common symbiotic pathway.

The agronomic benefits of rhizobial and AMF symbioses are attributed to an enhanced nutritional condition (due to N and P supplied by rhizobia and AMF), which, in turn, leads to increased photosynthetic rates and improved plant growth (Sa and Israel, 1991; Smith and Read, 2008). There is considerable interest in agronomy for the widespread use of AMF as 'bio-fertilizers' due to their benefits to plant nutrient acquisition, stress alleviation, and pathogen resistance. Recently, researchers reported markedly enhanced plant growth and yield while using two- or three-member synergistic associations of rhizospheric microbes with host plants. Previous studies considered the introduction of non-indigenous AMF species as ecologically benign, primarily due to the anticipated positive benefits (Azcon-Aguilar and Barea, 1997).

Therefore, to enhance crop productivity while maintaining agro-ecosystem sustainability, the focus has shifted from investigating plant-microbe interactions to plant-microbe-microbe interactions. In this context, the introduction of commercial AMF fertilizers represents an

attempt to address the productivity issues of low-P Saskatchewan soils in a more sustainable manner. Mycorrhizal fertilizer has been rather successful in horticultural systems (Azcon-Aguilar and Barea, 1997), but their usage in agricultural systems is not yet widespread. This can primarily be attributed to the non-replicability of growth chamber results in the field as well as variable responses of host crops, cultivars, and even climatic inoculation conditions. The differential effects on crops makes further investigation of the major factors influencing the success of an introduced AMF inoculant in a complex field environment imperative, particularly in cases of previously established farming/inoculant practices.

Commercial AMF inoculants typically contain a single AMF species (e.g., *Rhizophagus irregularis*). However, it is not known if this AMF enhances *Rhizobium* success, nor is it known if AMF success depends on the *Rhizobium* strain used. Given that Saskatchewan farmers typically apply *Rhizobium* inoculants, it is important to know if a second biological inoculant (i.e., AMF) will inadvertently affect the success of the N fixing association. Research suggests that in tripartite associations (i.e., AMF/*Rhizobium*/legume), indigenous AMF may be better suited to enhance crop growth parameters (Adekunbi, 2010), and it is possible that this is due to synergies related to the N_2 fixing association. The use of *Rhizobium* and AMF biofertilizers as an alternative/complementary source for N and P fertilizers in legume cropping systems is a promising technology. Before their widespread use can be recommended in various cropping systems, additional research needs to be carried out to assess the causes of differential results and to develop better management strategies for microbial consortium fertilizers.

The goal of this project was to investigate the interactions taking place during the legume-rhizobia-AMF tripartite symbiosis as well as its agronomical and ecological impact under actual field conditions in Saskatchewan. This can provide us with knowledge to develop and improve the use of biofertilizers for legume crops. The specific objective in field trial experiments was to represent and assess the effects of AMF inoculants and interactions with rhizobial inoculants on legume productivity, nutrient uptake, inoculation response, and biological N fixation under the actual environmental conditions experienced in the field. The secondary objective was to assess the impact of these interactions by examining differences in the AMF community composition of roots among the treatments. It was hypothesized that interactions between rhizobial and AMF inoculants would be synergistic in terms of consequent lentil and pea yield parameters. Two host plants and four experimental sites were chosen for trials between

2012 and 2013 to provide an overall representation of the primary legume crops and environmental conditions of Saskatchewan agricultural regions. The effects of AMF inoculation, *Rhizobium* inoculation, or dual applications in low P soil compared with uninoculated treatments were investigated. Three AMF application rates were tested, including the control, commercially recommended rate, and twice the recommended rate. The effect of treatments on plant agronomic properties, such as biomass (mid-season and harvest), nutrient acquisition, and biological N fixation, were evaluated.

The research presented in this thesis is in a traditional format that covers the impact of AMF and *Rhizobium* application in five legume cropping system field sites in Saskatchewan. The thesis is arranged in the following order: Introduction (Chapter 1); Literature Review of relevant publications (Chapter 2); Materials and Methodologies (Chapter 3); Results and Discussion (Chapter 4); and the Summary and Conclusion (Chapter 4).

Appendix A consists of additional supplemental data from the field trials, and Appendix B includes data from molecular experiments.

2. LITERATURE REVIEW

2.1 Rhizosphere, Mycorrhizosphere, and Microbial Interactions

The interactions between soil microorganisms and plant roots, as well as those among microorganisms themselves, create a microcosm at the soil-root interface of the plant, which is known as the 'rhizosphere'. Because it is intimately influenced by plant roots and high populations of microorganisms, the rhizosphere can be defined as the most active zone or as the 'living' zone of the soil in close proximity to the plant root (Glick, 1995; Nelson, 2004; Barea et al., 2005; Napoli et al., 2008). Plant roots and the rhizospheric microbial communities are related in a two-way relationship. The roots influence the microbes by depositing photosynthates into the rhizosphere (rhizodeposition) that are known to be species-specific, and the microorganisms concurrently influence plant physiology (Nelson, 2004; Napoli et al., 2008). Recently, rhizosphere soil has been operationally defined as the soil adhering to plant roots that can be separated with a moderate shake (Phillips and Fahey, 2008; Idris et al., 2009). Physically separating and distinguishing the rhizospheric soil from bulk soil is rather difficult (Hinsinger, 2005), but they inherently differ in the exhibition of different biological, chemical, and physical characteristics (Vessey, 2003; Barea et al., 2005; Hinsinger, 2005). The differences may be attributed to the presence of photosynthates and the intimate contact with the plant roots.

The rhizosphere microbial community is defined by the influence of plant assimilates (Hiltner, 1904). The rhizodeposits in the rhizosphere are rich in carbon, and they are characterized by increased microbial activity, which leads to a greatly enhanced microbial population relative to the bulk soil (Grayston et al., 1998). The stimulation of microbial communities in the rhizosphere provides a variety of benefits to the host plant, including enhanced nutrient solubilisation, growth stimulation, protection from infection by root pathogens, and the development of mutualistic symbioses. Due to the nearly universal colonising capability of AMF (80% terrestrial plants), the rhizosphere incorporates the fungal component of the symbiosis, resulting in the term 'mycorrhizosphere' (Rambelli, 1973; Linderman, 1988). The mycorrhizosphere is defined as the soil environment around the plant roots and AMF hyphae, where the AMF and soil bacteria interact. Since mycorrhizae and fungal hyphae are ubiquitous in natural soils, it could be argued that all soil should be included in the term 'mycorrhizosphere' (Johansson et al., 2004). The microbial interactions occurring in the mycorrhizosphere are

species-specific, and there is abundant literature testifying that rhizospheric microbionts are interdependent and that they influence each other (Rambelli, 1973; Bowen, 1980; De Oliveira, 1988; De Oliveira and Garbaye, 1989). However, most of the recent literature has mainly focused on the effects of mycorrhizosphere microbial communities on the extent of mycorrhizal colonization and on the mycorrhizal efficiency on host plant growth.

After the biotrophic colonization of the root cortex, the soil-borne mycorrhizal fungi develop an external mycelium, which acts like a bridge between the plants and the surrounding soil microhabitats in the rhizosphere. The mycorrhizal associations are mutualistic symbioses because of the interdependent and mutually beneficial relationship established between both partners. The host plant receives mineral nutrients via the fungal mycelium (mycotrophism), whereas the heterotrophic fungus obtains carbon compounds from the host's photosynthesis (Harley and Smith, 1983). Mycorrhizal symbioses are critical for the improvement of both plant fitness and soil quality through key ecological processes (Van der Heijden and Sanders, 2002; Smith and Read, 2008). Recently, it has been proposed that mycorrhizal symbiosis is a component of a microbial complex regulated by multitrophic interactions in the rhizosphere of host plant roots (Frey-Klett et al., 2007). Several plant root-microbe interactions can be formed from the specific interactions between groups of rhizospheric microbes as a series of developmental events, which are programmed based on molecular crosstalk between plant roots and microsymbionts. The microsymbionts are adapted to interact with and influence the rhizospheric niche, and their effects are mediated through a variety of mechanisms. Some of these interactions can act as 'bioprotection' against potential phytopathogens, while others can directly stimulate plant growth (Azcón-Aguilar and Barea, 1996; Barea 1997, 2000).

Plants in the Leguminosae family are capable of establishing mutualistic interactions with *Rhizobium* to fix atmospheric N in the plant's root nodules and with AMF, which can increase the uptake of phosphorus and other nutrients from the soil (Allen and Allen, 1981; Harley, 1971; Smith, 1980). The interactions between bacteria and AMF are potentially beneficial, including interactions where plant growth-promoting bacteria (PGPR) (Meyer and Linderman, 1986; Von Alten et al., 1993; Kloepper, 1994, 1996), including N_2 fixing bacteria (Secilia and Bagyaraj, 1988; Biro et al., 2000), are involved. Some bacteria are known to affect AMF germination and growth rates, (Mosse, 1959; Daniels and Trappe, 1980; Mayo et al., 1986; Carpenter-Boggs et al., 1995) so the beneficial impact to the plant could be through the AMF association. Other

bacteria are known to directly influence plant physiology (e.g. by increasing root cell permeability). Apart from directly interacting to beneficially influence the mycorrhizal relationship and/or plant growth (Linderman, 1988, 1992; Garbaye, 1994; Vivas et al., 2003), specific bacteria with AMF may create a more indirect synergism that supports plant growth (Barea, 1997), including nutrient acquisition (Barea et al., 2002b), inhibition of plant pathogenic fungi (Budi et al., 1999), and enhancement of root branching (Gamalero et al., 2009).

The whole AMF/rhizobia interaction process works both ways, so AMF are also known to affect the composition of bacterial communities present in and around it (Artursson et al., 2005). This fact has been attributed to the chemical composition of plant root exudates produced as a result of the establishment of an AMF infection, and these are known as nutrient sources to some specific groups of bacteria in the mycorrhizosphere, thus favouring their growth over others (Harley and Smith, 1983; Linderman, 1992; Azcón-Aguilar and Bago, 1994; Smith et al., 1994; Barea, 1997, 2000; Gryndler, 2000; Linderman, 2000). However, the above impact can also be related to more direct interactions such as competition for nutrients and ecological needs (Christensen and Jakobsen, 1993). Some recent studies further established the fact that AMF-legume-*Rhizobium* interactions are highly specific and a lot more than just nutrient exchange occurs. For instance, the interactions take place more at the cellular level with specific signalling and feedback mechanisms. Studies show that some bacterial species respond to the presence of certain AMF (Andrade et al., 1997; Artursson et al., 2005), which suggests a high degree of specificity between bacteria associated with AMF. One possible explanation for this kind of specific interaction is related to species-specific fungal exudates.

Understanding the complex interactions taking place in the mycorrhizosphere is crucial to improving agricultural productivity and maximizing ecological benefits provided by microbial interactions. The mycorrhizal literature of conventional agriculture (Bagyaraj, 1992) has traditionally focused on the potential of mycorrhizal fungi to improve crop yield and their potential as a fertilizer. The close relationship between the mycorrhizal soil mycelia and the soil biota is well known (Curl and Truelove, 1986; Linderman, 1988).

2.2 Arbuscular Mycorrhizal Fungi

The term ‘mycorrhiza’ stands for ‘fungus-root’ as derived from the Greek words ‘mycos’ (fungus) and ‘rhiza’ (root). It was coined by a German botanist A.B. Frank (1885) to represent

the mutualistic association existing between the fungi and roots of woody plants under various habitat systems. The particular association he reported was later termed 'ectomycorrhizae', and he further went on to hypothesize the exact nature and mechanism of the mycorrhizal association. More than half a century later Mosse (1953) identified and cultured an AMF species with a strawberry plant as host, and it was named *Endogone mosseae* (renamed *Funneliformis mosseae*) (Koide and Mosse, 2004; Kruger et al., 2011). A. B. Frank was also the first to propose that mycorrhizal fungi are capable of extracting nutrients, particularly N, from organic matter (Frank, 1885, 1887). Almost a century later, research demonstrated that mycorrhizal associations could break down cellulose, hemicelluloses, and humic polymers, thereby providing plants with additional N sources (Durall et al., 1994; Read, 1987).

Two types of mycorrhiza are distinguished by the colonization procedure and host preference. Ectomycorrhizae are primarily formed on forest trees mostly by basidiomycetes and some ascomycetes. They form an external lightly interwoven fungal mantle and an intercellular hyphal network in the host's root cortex called 'Hartig's net'. In contrast, endomycorrhizal fungi penetrate root cortical cells and form specialized feeding hyphae called arbuscules, or they form large swollen food-storing hyphal swellings called vesicles (Agrios, 2005). Most plant species set up an association with AMF, and it is one of the most important symbioses on earth, linking the root and the soil systems (Koide and Mosse, 2004). Arbuscular mycorrhizal fungi belong to the phylum Glomeromycota and the order Glomales (Schüßler et al., 2001). Fossil evidence places the appearance of both AMF and plants at almost 400 million years ago (Parniske, 2000). More than 80% of plant species can be colonized by AMF, yet relatively few fungal species (~120) from a restricted order, the Glomales, are actively involved in colonizing plant roots. Most of the major plant families associated with agricultural systems are able to form AMF associations (Barea et al., 1993). Few vascular plant species, belonging mainly to the Cruciferae, Chenopodiaceae, Cyperaceae, Caryophyllaceae, and Juncaceae families, are evolutionarily incapable of forming mycorrhizal associations.

The arbuscular mycorrhizal association is primarily a mutualistic symbiosis between plant and fungus that is localized in the arbuscules, in which energy moves from plant to fungus, and inorganic resources move from fungus to plant. Arbuscular mycorrhizal symbioses can be distinguished from other fungal associations due to several criteria, including the mutualistic nature of mycorrhizas and the modification of host physiology due to symbiosis establishment.

The host plant receives mineral nutrients via the fungal mycelium (mycotrophism), whereas the heterotrophic fungus obtains carbon compounds from the host photosynthates (Harley and Smith, 1983).

The establishment and development of plant-AMF involves alterations in host physiology, and it consists of a series of interactions that lead to the integration of both organisms via continuous molecular 'dialogue' through recognition and acceptance signals (Gianinazzi-Pearson, 1997; Vierheilig and Piché, 2002; Matusova et al., 2005). The initial signals released by the plant are the strigolactones, which are responsible for inducing hyphal branching in AMF spores, promoting their growth, and increasing their contact with the host root (Buee et al., 2000; Akiyama and Hayashi, 2006). In response to the release of strigolactones, the fast branching fungal hyphae release a diffusible signalling molecule identified as a 'Myc' factor, which activates the promoter of the symbiosis-related gene *ENOD11* in roots (Kosuta et al., 2003; Maillet et al., 2011). At the onset of infection establishment 'Myc factors' express the symbiotic genes in plants. The genetic activation sets the structural and physiological alterations in the host plant into motion. The contact between fungal hyphae and the root epidermis triggers the formation of the fungal infective structure referred to as the 'appressorium', which further signals the epidermis to start cellular reorganization of the receptive cell. The nucleus of the underlying cell creates an aggregation of microtubules, actin microfilaments, and ER cisternae, organized into a finger-shaped structure. The pre-penetration apparatus (PPA) is projected into the cell, thereby establishing a symbiotic mutualism with the host plant (Fig. 2.1) (Akiyama et al., 2005; Parniske, 2008; Feddermann et al., 2010). Symbiosis establishment ends with the formation of a specialized intracellular structure, the arbuscule, a symbiotic interface to exchange signals, nutrients, and carbohydrates with the host. Arbuscules are constructed via the elaborate cellular reorganization of vacuoles, cytoskeleton, and plastids, and they are surrounded by peri-arbuscular membranes, which are extensions of cell plasmalemma (Genre and Bonfante, 1998; Fester et al., 2001).

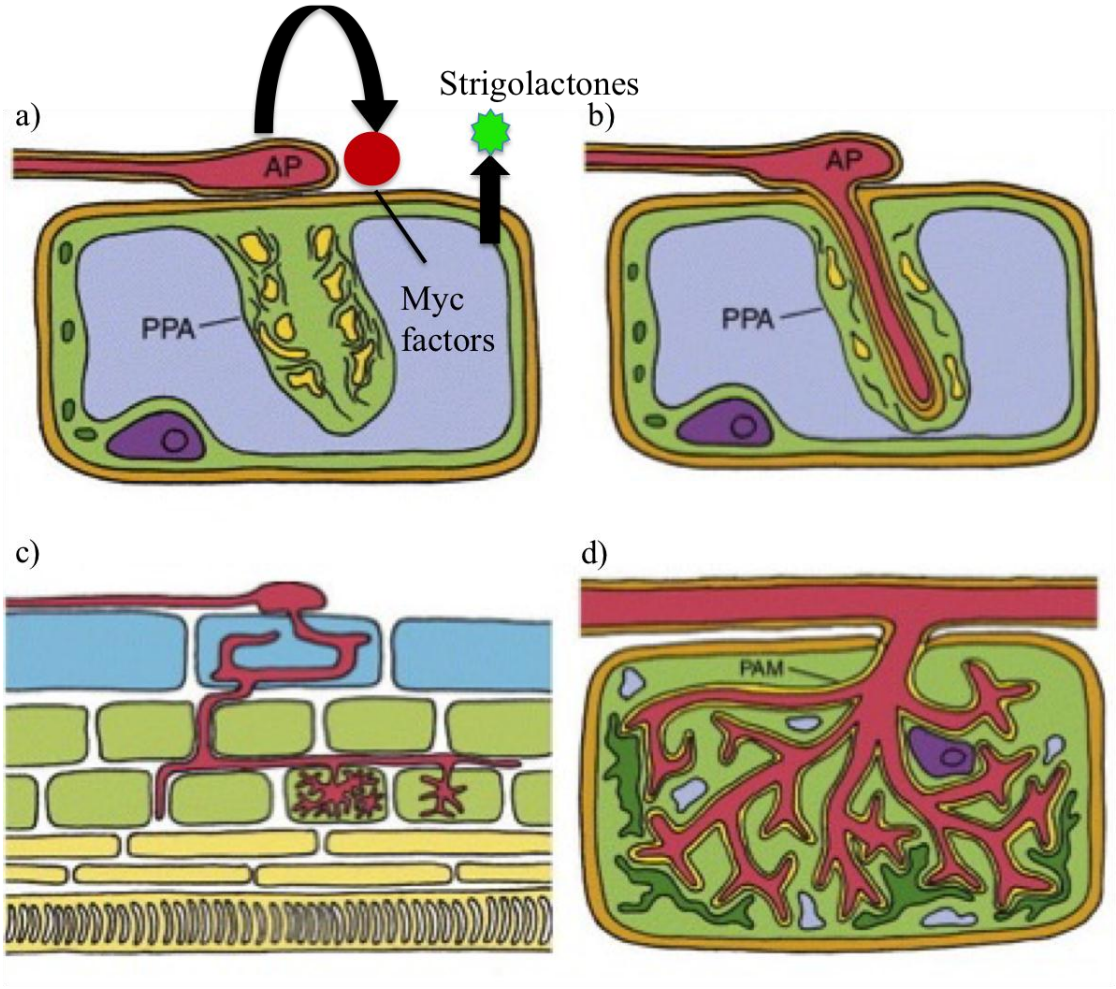


Fig. 2.1. Establishment of mycorrhiza: a) molecular signalling between the fungal appressorium (AP) and the plant cell; b) formation of the pre-penetration apparatus (PPA) via cellular reorganization; c) colonization of the root cortex and intercellular growth of the fungal hyphae; and d) a fully developed arbuscule enveloped by the peri-arbuscular membrane (PAM) in a plant cell. Figure has been modified, with permission from Reinhardt (2007).

The arbuscules are primarily highly ramified hyphae with fine terminal tips, providing them with a higher surface-to-volume ratio and higher efficiency for nutrient transfer compared to normal hyphae (Dickson and Kolesik, 1999). The establishment of arbuscules creates an elaborate cellular mechanism for nutrient transfer in the infected cells, which leads to physiological modification in the host plant. The phosphate transporters in the PAM are induced and the arbuscules are activated to take up nutrients, which enhance the overall growth of the host (Rausch et al., 2001). The arbuscules also function as the AMF carbohydrate supply sites for the plant (Harrison et al., 2002).

Interestingly, the plant genes responsible for the establishment of successful AMF symbioses are also expressed during *Rhizobium* nodule organogenesis and N fixation. The inducible genes control the signalling pathway referred to as the CSP (Demchenko et al., 2004; Parniske, 2004; Akiyama and Hayashi 2006; Parniske, 2008; Feddermann et al., 2010). Mycorrhizas and *Rhizobium* are both intracellularly hosted, but their pathogenesis programs are different. Both symbioses use a common signalling pathway that activates the transcription factor CYCLOPS by phosphorylation and leads to cell division in the roots to form nodules. In contrast, the expression of CYCLOPS during mycorrhiza establishment does not involve any cell division.

In AMF-plant symbiosis, mycorrhizas translocate nutrients to the plant through the mycelial network, with a return of 10% and 23% of host photosynthates (Snellgrove et al., 1982; Kucey and Paul, 1982; Koch and Johnson, 1984; Jakobsen and Rosendahl, 1990). The mycorrhiza symbiosis is a key to productivity, and it is rare to find a situation where AMF do not have a significant ecological presence. They account for 5–50% of the biomass of soil microbes (Olsson et al., 1999). The biomass of AMF hyphae may amount to 54–900 kg ha^{-1} (Zhu and Miller, 2003), and some products formed by them such as various exudates may account for another 3000 kg (Lovelock et al., 2004). The external mycelium attains as much as 3% of the root weight (Jakobsen and Rosendahl, 1990). With the numerous AMF benefits, it is apparent that AMF play a key role in ecosystem processes. In light of the increasing evidence about the benefits of AMF-plant symbiosis in various ecosystems and crops (Barea et al., 2005), ascertaining the effectiveness and feasibility of manipulating this beneficial association, particularly in agricultural production systems, is of importance.

2.2.1 Role of AMF in plant growth and soil ecology

The mycorrhizal symbiosis has received attention for its ability to enhance the host plant's uptake of relatively unavailable biological nutrients (particularly P) and several micronutrients. Several studies show the contributions of AMF towards the enhancement of physiological aspects of plants such as nutrient acquisition, root lengthening, and stress alleviation (Kapulnik and Douds, 2000; Hodge, 2000; Koide and Kabir, 2000; Rillig, 2004; Richardson et al., 2009). AMF symbioses primarily increase the supply of mineral nutrients to the plant, particularly those that are relatively immobile or those that are low in concentration, including phosphate, ammonium, zinc, and copper (Koide and Kabir, 2000). Improved P uptake is the best-documented benefit of mycorrhizal associations for the plant host. Several researchers postulated that the plant P status is the controlling factor in plant-AMF relationships (Smith and Read, 2008; Graham, 2000), and that mycorrhizas may benefit plants by increasing the availability of P from non-labile sources. It is widely accepted that mycorrhization enables increased spatial exploitation of the soil environment by plant roots via fungal hyphae (Marschner, 1995). Linderman (1992) suggested the possibility that, when acquiring P from the soil, the extraradical hyphae of mycorrhizal fungi can increase the 'competitive ability' of the mycorrhized plant's root system compared to soil microorganisms.

Research has shown that AMF can function as more than just an extension of the plant's root system. Mycorrhizal associations provide the host plant with a very effective pathway (the AMF pathway) for the rapid delivery of P to the cortical cells within the root, bypassing the direct uptake method. The well-developed mycorrhizal hyphal network absorbs orthophosphate (P_i) through fungal high affinity P_i transporters (P_iTs) (Marschner, 1995). Plants capable of forming mycorrhizas carry specific AMF-inducible PiT genes (different from regular P_iT genes involved in direct P uptake), which are exclusively expressed in the colonized cortical cells (Bucher, 2007; Javot et al., 2007). P_i taken up by the hyphal network is released into the interfacial apoplast of the colonized cells, and plant P_iTs are involved in the uptake from that point. Additionally, H^+-ATPases energize the plant plasma membrane surrounding the intracellular fungal structures, facilitating active P_i uptake (Smith and Read, 2008). The individual fungal hyphae have much smaller diameters than roots, allowing access to narrower soil pores, and increase the soil volume explored up to several centimetres from the root surface,

which can markedly extend the depletion zone (Drew et al., 2003; Javot et al., 2007; Smith and Read, 2008; Schnepf et al., 2011).

Mycorrhizas are reportedly important for the nutritional improvement of other macronutrients such as N and potassium (K). Researchers reported that fine fungal hyphae are better able to penetrate decomposing organic material for recently mineralized N (Hawkins et al., 2000; Hodge, 2003). AMF are also known to contribute to the uptake of N in forms of nitrate (NO_3^-) and relatively immobile ammonium (NH_4^+) in acidic and organic soils, but more work is required to fully understand the mechanisms involved (Hodge et al., 2001; Read and Perez-Moreno, 2003). In legumes, AMF can indirectly affect N availability because enhanced uptake of P is important for N_2 fixation, which is an ATP-intensive process (Smith and Read, 2008). Higher concentrations of K are seen in mycorrhizal plants compared to non-mycorrhizal plants. Regarding plant growth, increased K concentrations can be a consequence of increased P availability, because the P and K uptake pathways are intimately related (Bressan et al., 2001; Liu et al., 2002). AMF can be important in the uptake of other nutrients, such as zinc (Zn), copper (Cu), iron (Fe), N, K, calcium (Ca), and magnesium (Mg), and the uptake of these nutrients has been enhanced under mycorrhizal conditions (Clark and Zeto, 2000; Ryan and Angus, 2003; Smith and Read, 2008).

Researchers observed both higher nutrient and water uptake in water- and salinity-stressed mycorrhizal plants than in non-mycorrhizal plants (Al-Karaki and Clark, 1999; Feng et al., 2002; Srivastava et al., 2002; Mohammad et al., 2003). This is attributed to the physiological changes that cause improved stomatal regulation, osmotic adjustment of the host, and improved access of soil pores due to hyphae, which enables enhanced water uptake (Auge´, 2001, 2004). In addition, AMF and their products (e.g. glomalin) directly contribute to soil structural formation, and the external AMF mycelium forms water-stable aggregates necessary for good soil tilth (Miller and Jastrow, 2000; Jeffries and Barea, 2001; Jeffries et al., 2002; Rillig and Mummey, 2006).

Mycorrhizal associations are known to offer protection against plant pathogens, and to interact with heavy metals/micronutrients. Moreover, they can also restore the nutrient uptake equilibrium that is misbalanced by heavy metals (Siqueira et al., 1999; Carneiro et al., 2001). Researchers observed alleviation of aluminium (Al) and manganese (Mn) toxicity in mycorrhizal

plants compared to non-mycorrhizal plants (Nogueira et al., 2004; Rufyikiri et al., 2000). AMF also play a potential role in the monitoring of site toxicity (Weissenhorn et al., 1993, 1995; Gucwa-Przepióra and Turnau, 2001) and in the efficiency of restoration techniques (Orłowska et al., 2002). Mycorrhizal parameters are used as biological indicators for the biomonitoring of soil quality (Lovera and Cuenca, 1996; Haselwandter, 1997; Jacquot et al., 2000). Levels of AMF colonisation of grasses in polluted soils correlate with heavy metal contamination (Mikanovμ et al., 2001). Furthermore, AMF associations are known to possibly be used as both biocontrols and bioremediation agents (Bethlenfalvay and Linderman, 1992; Gianinazzi and Schüepp, 1994; Gianinazzi et al., 2002). The protective actions of AMF symbiosis include: the improvement of plant vigour; damage compensation; competition with pathogens for photosynthates or for colonization/infection sites; induction of changes in the morphology/anatomy of the root system; induction of changes in mycorrhizosphere populations; and activation of plant-defence mechanisms (Pozo et al., 2002; Azcón-Aguilar et al., 2002; Barea et al., 2005). By increasing the plant nutrient content and influencing rhizodeposition by the plant, AMF modify plant growth and alter rhizospheric processes (Richardson et al., 2009). As a result, AMF play crucial roles in the soil system. Rhizosphere interactions occur between AMF and other soil microorganisms, such as N fixing bacteria and plant growth-promoting rhizobacteria and they affect plant nutrient balances (Paula et al., 1992).

2.2.2 Mycorrhizal technology and biofertilizers

The beneficial attributes of AMF in enhancing plant growth and health make them important components of the ecosystem (Barea et al., 2005). Inoculating plants with AMF inoculum is an emerging innovative method for the enhancement of crop efficiency. Using AMF in agriculture can decrease the use of chemical fertilizers, thereby decreasing chemical pollution in soil water (Giovannetti, 2001). Direct inoculation of either the host plant or the soil with AMF has been shown to increase P uptake, and in some cases it increased the yield under actual field conditions in AMF-dependant crops (Torres-Barraga´n et al., 1996; Kahiluoto and Vestberg, 1998; Al-Karaki, 2002; Mohammad et al., 2004; Al-Karaki et al., 2004; Douds et al., 2005). Inoculation experiments show unpredictable and inconsistent responses among different AMF and host species as well as soil P levels, where the range varies from significantly positive to significantly negative (Hamel et al., 1997; Xavier and Germida, 1997; Al-Karaki, 2002; Douds

and Reider, 2003; Klironomos, 2003). There are three essential parameters that affect the performance of the AMF inoculum in agriculture: appropriate AMF species (Estaun et al., 2002), the quality of AMF inoculum (Von Alten et al., 2002), and their ecological properties (Feldman and Grotkass, 2002). Selecting an AMF inoculum species that has a broad range of host responses and effects is a complicated task, because different AMF species show different effects and effect levels among a variety of hosts under varied conditions. This can explain the reason that some inoculants failed the 'field test' even though they were successful in colonizing the host. Several researchers argue that identifying an effective AMF/host/inoculation combination does not translate into a successful field inoculum because the problem of competition with the native soil AMF remains. Native AMF are more adapted to the soil environment because they co-evolved with the host crops, and they may out-compete the introduced strains or negate the benefits of the inoculation (Harinikumar and Bagyaraj, 1996; Izaguirre-Mayoral et al., 2000; Klironomos, 2003). Kahiluoto and Vestberg (1998) reported a yield depression upon introducing AMF inoculum under field conditions. They concluded that the native AMF population was sufficiently effective, and that the introduction of a new strain caused a yield decline. Moreover, the rhizosphere of a crop plant is usually colonised with a variety of other soil microorganisms, some of which are synergistic whereas others might be antagonistic towards both AMF and the plant. Therefore, the study of multi-microbial interactions in the rhizosphere of microplants may be a very useful approach for developing the understanding of AMF management in plant production systems (Cordier et al., 1999). The recent development of molecular probes that are able to differentiate AMF within roots and soil (Tuinen et al., 1998; Jacquot et al., 2000; Jacquot-Plumey et al., 2001) provides new biotechnological perspectives to define the population biology and management strategies for the use of these symbiotic microbes in agriculture.

2.3 Nitrogen-Fixing Bacteria

The symbiotic legume-rhizobial interaction in the rhizosphere is one of the most researched and well-characterized plant-microbe interactions in environmental and agricultural sciences. The N_2 fixing *Rhizobium* symbiosis is essentially restricted to legumes (Fabaceae), with the exception of member of the *Parasponia* genus (Cannabaceae). Cannabaceae and Fabaceae diverged ~100 million years ago, and are represented by the split of Fabales and the Fagales,

Cucurbitales, and Rosales orders (Wang and Qiu, 2009). Phylogenetically, it has been established that the common ancestor of legumes was not an N fixing system but an AMF association (Parniske, 2000; Provorov et al., 2002). The ability to nodulate likely evolved independently in this family, and been lost in several genera when the availability of mineral N increased.

The colonization process starts with the secretion of flavonoids by the roots of the host plant, which attracts the entry of the appropriate rhizobial species. Rhizobia produce species-specific complex sugar derivatives, called lipo-chitooligosaccharides (Nod factors), which are perceived by plant LysM-like receptors. These activate a signal transduction pathway required for the invasion process and the subsequent development of a new root organ called the nodule (Riely et al., 2004, Geurts et al., 2005), which causes root hair deformation, branching, and/or curling. This communication results in the rhizobia entering the root by way of an infection thread. As the infection thread elongates, root inner cortical cells are induced to divide, and these cells become the nodule primordium. During root-nodule organogenesis, a nodule primordium is formed from previously differentiated cortical cells, and rhizobia are guided to this primordium in a host-controlled manner. The thread enters these cells and releases the rhizobia, which remain confined within vesicles bound to the plant-derived peribacteroid membrane (Gage and Margolin, 2000).

Rhizobia remain outside the plant cytoplasm, and are engulfed in a symbiosome membrane that functions to regulate nutrient exchange between the partners. Nodules arise from re-differentiating root pericycle and cortical cells, and they are later invaded by Rhizobia (Hirsch, 1992). After further growth and differentiation of the nodule, the rhizobia start converting N from the air into ammonia, which is exported to the plant as amino acids. In exchange, rhizobia import C from the plant. This nutrient exchange requires coordination of transport processes of both partners (Lodwig et al., 2003). The RL symbiosis also requires feedback mechanisms, so the symbiosis can be limited at times of sufficient N supply to the plant (Caetano-Anollés and Bauer, 1988). N fixing bacteria improve the bioavailability of N to plants, and this is enhanced by the presence of mycorrhized roots (Barea et al., 2002c). *Rhizobium* strains are known to colonize the rhizosphere of non-legume hosts, where they establish positive interactions with AMF (Galleguillos et al., 2000).

2.4 AMF-Legume-Rhizobia Interactions

When one organism interacts mutualistically with each of two others, various new phenomena may result. The two might compete for the services of the third, particularly if the two receive essentially the same benefits from it. On the other hand, the two mutualists might indirectly help each other by increasing the growth and/or density of their shared mutualist, creating a new indirect mutualism of the 'friends' friends' type (Boucher et al., 1985). Finally, the two mutualists might come to directly interact with each other, either positively or negatively, in addition to their indirect interactions via the third mutualist (Thompson, 1982). Just as competition may involve either direct interactions (interference) or indirect ones through interactions with a resource species (exploitation), the newly created mutualism may also be direct or indirect.

Asai (1944) pioneered the view that root nodulation by *Rhizobium* can be dependent on the formation of mycorrhiza. Recent developments regarding the abundance of the AMF in nodulated legumes, and its role in improving both nodulation and *Rhizobium* activity within the nodules, are recognized (Barea et al., 2002a, 2002b; Provorov et al., 2002), which has given rise to the concept of a tripartite symbiosis among legume-mycorrhiza-rhizobia. The interaction between the two endosymbionts actually occurs at the level of colonization and/or at the functionality (nutritional) level (Barea and Azcón-Aguilar, 1983). As described by Hayman (1986) and Mosse (1986), because of the relatively high P demand for nodule formation, it is obvious that a major benefit of AMF on the symbiotic role of rhizobia must be the P supplied by the fungus. However, nutrients other than P (e.g. Zn, Cu, Mo, Ca, etc.) can affect both the infectivity and the symbiotic effectiveness of *Rhizobium*. Therefore, the enhanced uptake of these elements by the AMF symbiosis may also be involved in the interactions. Conversely, fixed N supplied by N_2 fixation resulting from rhizobial activity can be critical for maintaining a balanced physiological status in the plant, which is important for AMF formation and function. In addition, there is a high requirement for fixed N by AMF to synthesize chitin, the main component of its walls. Therefore, nodulation and AMF formation appear to be mutually supportive. Production of extracellular polysaccharides by two PGPRs, *Azospirillum* and *Rhizobium*, significantly enhanced the attachment of bacterial strains to mycorrhizal roots and AMF structures. These polysaccharides are thought to significantly influence the movement of

bacterial strains into new rhizospheres, and they are important for the effective production of microbial inoculums (Bianciotto et al., 2001).

There are a large number of bacteria, including PGPR and rhizobia, called mycorrhiza helper bacteria (MHB), which are known to promote the activity and development of AMF (Frey-Klett et al., 2007; Richardson et al., 2009). They are usually fungus-specific but not plant-specific (Rillig et al., 2005). Studies attribute such specificity to the spore size and the roughness of the spore surface (Bharadwaj et al., 2008). Moreover, they are known to influence spore germination by affecting the spore wall (Maia and Kimbrough, 1998; De Boer et al., 2005), and they stimulate spore germination by producing stimulants such as CO_2 (Carpenter-Boggs et al., 1995).

Additionally, AMF can also alter the combination of bacteria in the rhizosphere through competition for soil nutrients (Christensen and Jakobsen, 1993). Researchers observed that the association of some bacteria with AMF is specific (Artursson et al., 2005), indicating that there is some kind of communication between the bacteria and AMF that is stimulated by fungal exudates (Artursson et al., 2006). The significance of bacterial attachment to the AMF hyphae and whether it can affect hyphal growth has not been determined. According to Bianciotto et al. (1996), the attachment intensity of some strains of *Rhizobium* and *Pseudomonas* to AMF germinating spores and hyphae under sterilized conditions differed depending on the bacterial strains; however, the level of specificity was not recognized. For PGPRs, their adherence to AMF is determined by the formation of biofilms, which are extracellular matrices, which are produced by bacteria and the bacteria themselves (Seneviratne et al., 2009).

The interactions between AMF and soil bacteria, particularly PGPR (Von Alten et al., 1993; Kloepper 1996) and N fixing bacteria, are typically beneficial to the host plants. The dual inoculation of AMF and *Rhizobium* causes synergistic beneficial effects, and they act as biofertilizers for crops (Champawat and Pathak, 1993; Kumar et al., 1999; Xavier and Germida, 2002). They also play a key role in natural ecosystems and influence plant productivity, plant nutrition, and plant community structure. The dual symbiosis with AMF and rhizobia is crucial for legume growth within plant communities (Rao et al., 1986; Cleveland et al., 1999; Van der Heijden et al., 2006). In many experiments co-inoculation with both symbionts resulted in higher plant biomass and better N and P acquisition, but these effects were dependent on the specific

symbiont (Azcon et al., 1991; Xavier and Germida, 2002). Tripartite symbiotic associations were more effective than AMF or *Rhizobium* inoculation alone with respect to the uptake of N and P by plants (Azcon et al., 1991; Linderman, 1992; Saxena et al., 1997).

For N_2 fixing rhizobia, the mycorrhizal and root nodule symbioses are typically synergistic with regard to the infection rate, their impact on mineral nutrition, and the growth of the plant (Barea, 1997; Barea et al., 2002). Toro and colleagues (1998) showed that N_2 fixation rates in mycorrhizal alfalfa plants inoculated with *R. meliloti* were higher than the corresponding rates in non-mycorrhizal plants using the based on the natural ^{15}N abundance method. AMF are known to positively affect N_2 fixation by influencing the energy producing pathways through enhanced P uptake (Mortimer et al., 2008). The hormonal effects produced due to root and nodule mycorrhization are also known to affect N_2 fixation in tripartite symbioses (Franzini et al., 2010). Despite all of the added benefits of the synergistic effects that are seen in tripartite symbioses, the efficiency of the interaction is vastly dependent upon the plant variety, strains of the AMF and *Rhizobium* used, the related interactions, and the growth stage of the host plant (Marulanda et al., 2006; Mortimer et al., 2008).

2.4.1 Tripartite symbiosis and molecular pathways

In natural conditions, AMF and *Rhizobium* are known to colonize the root almost simultaneously, and they are non-competitive for infection sites. However, if the inoculations are added chronologically, the previous one can depress the development of the latter (Bethlenfalvay et al., 1985). This has been mainly attributed to competition for the limited host photosynthates. In such cases, AMF usually show a competitive advantage for carbohydrates over *Rhizobium* (Brown and Bethlenfalvay, 1988).

Phylogenetic and molecular interaction patterns of N_2 fixing and mycorrhizal microbe-plant symbioses suggest a common developmental program for these associations (Parniske, 2000). A common ancestral plant-fungal interaction has been proposed, since rhizobia-legume symbiosis appears much later than AMF associations (Sprent, 1994; Redecker et al., 2000; Provorov et al., 2002), it has been hypothesised that the cellular and molecular events occurring during legume nodulation may evolve from those already established in the AMF symbiosis (Gianinazzi-Pearson, 1997). In fact, legume-rhizobia symbiosis seems to be evolved from a set

of pre-adaptations during co-evolution with AMF (Provorov et al., 2002). The possibility that some plant genes can modulate both types of legume symbiosis is a research field of current interest (Ruiz-Lozano et al., 1999).

The tripartite endosymbiosis in a legume plant is established as a result of signal exchange, in which there is mutual recognition of diffusible signals produced by plant and microbial partners. The signals produced by the microbial partners are LCOs (Maillet et al., 2011). These LCOs are perceived via LysM receptors, and they activate CSP, which controls the conserved RL and AMF symbioses (Chen et al., 2007, 2008, 2009; Banba et al., 2008; Kouchi et al., 2010). The rhizobial LCOs are called Nod factors (De'Haeze and Holsters, 2002). Recent work has established that an AMF, *G. intraradices*, produces LCOs that activate the CSP, leading to the induction of gene expression and root branching in *M. truncatula* (Gough and Cullimore, 2011). Research confirms that *G. intraradices* secretes symbiotic signals that are a mixture of sulphated and non-sulphated LCOs, which stimulate the formation of AMF in plant species from diverse families (e.g. Fabaceae, Asteraceae, and Umbelliferae) (Maillet et al., 2011).

3. MATERIALS AND METHODS

3.1. Field Experiments

3.1.1 Study sites

Experimental plots were established in spring 2012 and 2013 in commercial farm fields at five different locations in the agricultural region of Saskatchewan, Canada. In 2012, the locations were Kelvington (east of Saskatoon) and Stewart Valley (north of Swift Current); in 2013, the locations were Stewart Valley, Outlook and Pampbrun, SK. The legal land locations and other details are given in Table 3.1. Sites were under cereal cultivation in the previous years at all locations. Precipitation and temperature data for the growing season along with historical climatic data were collected from the nearest Environment Canada research stations.

The soil at Kelvington was a thin Orthic Black Chernozem of the Oxbow Association. These soils are loamy in texture and occur on a gently sloping topography, typical of the northern agricultural region of Saskatchewan (Saskatchewan Soil Survey, 1995). The soil at Stewart Valley was an Orthic Brown Chernozem of the Sceptre Association with undulating topography. The soil and landscape was typical of the South Saskatchewan River Valley area (Ayres et al. 1985). The trial site at Outlook was a thin Orthic Dark Brown Chernozem of the Asquith Association of a loamy texture (Ellis et al. 1970). The soil at Pampbrun was an Orthic Brown Chernozem of the Fox Valley Association (Saskatchewan Soil Survey, 1989; Soil Classification Working Group, 1998).

Soil samples for nutrient analyses were taken with a soil auger prior to seeding, at depth increments of 0 to 15 cm at four random locations of the trial area. Sub-samples from the 0 to 15 cm depth were bulked and stored at 4°C for most probable number (MPN) assay of soil AMF propagules. Samples from each depth were bulked, dried and sent to ALS Laboratory Group (Saskatoon, SK) for pH, cation exchange capacity (CEC), and macronutrient analysis (Table 3.1).

3.1.2 Soil characterization and meteorological data

In the early spring of 2012 and 2013, initial soil sampling was done and basic soil characteristics were measured and are summarized in Table 3.1.

Table 3.1. Study site locations and initial soil characteristics in the upper 15 cm of the soil profile.

	2012		2013		
Site	Kelvington	Stewart Valley	Stewart Valley	Outlook	Pampbrun
Location	SW 30 36 12 W2	NW 19 19 12 W3	NW 19 19 12 W3	SW 15 29 8 W3	NW 11 11 11 W3
Soil Zone	Black	Brown	Brown	Dark Brown	Brown
Soil Texture†	Loam	Clay	Clay	Loam	Clay Loam
pH‡	7.8	8.2	8.2	8.5	8.3
EC (mS m^{-1})§	0.25	0.24	0.18	0.25	0.17
Soil Test N (kg ha^{-1})¶	12.1	9.9	8.8	12.1	5.5
Soil Test P (kg ha^{-1})#	3.3	11.1	8.8	34.1	38.5
Soil Test K (kg ha^{-1}) #	759	>1199	>1199	354.2	>1199
Soil Test S (kg ha^{-1})††	20.9	9.9	8.8	>52.8	9.9
MPN (per 100 g soil)	117	85	88	83	74

† Soil textures were determined using USDA texture triangle.
‡ pH of a 1:2 (soil: water) extract.
§ EC (electrical conductivity of a 1:2 (soil: water) extract.
¶ Available nitrate and nitrite were extracted from the soil using a dilute calcium chloride solution.
# Plant available phosphorus and potassium were extracted from the soil using Modified Kelowna solution.
†† Plant available sulfur in the soil was extracted with a weak calcium chloride solution.

The meteorological data (daily mean temperature and rainfall/snow) for the study period of 2012 and 2013 and the 30-year long-term average of all the experimental sites are reported in Table 3.2. In 2012, Kelvington and Stewart Valley experienced average cropping season temperatures except in May, which was cooler than the historic average (Table 4.1.). Both of the sites also experienced higher precipitation than the 30-year average precipitation for the early part of the growing season (April-June) and the trend continued until August at Kelvington. All the sites in 2013 experienced historically colder temperatures in April, but the temperatures were closer to the 30-year normal levels later in the season (May-September). It was a relatively dry spring (April-May) at all the sites in 2013.

Table 3.2. Weather data for the study sites. Data from closest Environment Canada meteorological stations.

Site	Year	Months					
		April	May	June	July	August	September
		Daily mean temperature (°C)					
Kelvington	2012	2.6	9.6	15.2	18.9	17.1	12.4
	30-year average†	2.8	10.7	15.9	17.5	16.8	10.9
Stewart Valley	2012	5.1	9.4	15.5	20.0	19.0	13.8
	2013	-0.9	12.6	15.5	16.8	19.2	15.2
	30-year average	5.2	10.9	15.4	18.5	18.2	12
Outlook	2013	-1.5	12.9	15.9	17.5	18.8	15.6
	30-year average	5.3	11.5	16.1	18.9	18	12.3
Pambrun	2013	-0.9	12.6	15.5	16.8	19.2	15.2
	30-year average	5.2	10.9	15.4	18.5	18.2	12
		Total precipitation (mm)					
Kelvington	2012	24.8	55.2	112.3	97.8	68.1	12.6
	30-year average	26.7	42.9	54.3	76.7	52.4	38.7
Stewart Valley	2012	63.0	98.3	107.0	17.2	8.2	4.9
	2013	11.8	11.2	103.0	50.4	13.5	42.8
	30-year average	19.9	48.5	72.8	52.6	41.5	34.1
Outlook	2013	9.9	12.7	73.5	28.0	28.8	35.7
	30-year average	21.6	42.6	63.9	56.1	42.8	34.1
Pampbrun	2013	11.8	11.2	103.0	50.4	13.5	42.8
	30-year average	19.9	48.5	72.8	52.6	41.5	34.1

† 30 years average of daily mean.

3.1.3 Experimental design and sampling

In 2012, two factor factorial field experiments were conducted at Kelvington and Stewart Valley using a randomized complete block design with four replicates. The first factor was AMF and P was the second factor. The total number of experimental units per site was 24 with 6 treatments and individual plot size of 10 × 1.5 m. Treatments included an AMF commercial inoculant MYKE®PRO GR (Premier Tech, Quebec, Canada) applied at three rates (0, 7.5 kg ha^{-1} and 15 kg ha^{-1}) alone and in combination with 16.8 kg P_2O_5 ha^{-1} as mono ammonium phosphate (11-52-0). MYKE®PRO GR is a granular mycorrhizal inoculant recommended for seed row application and carries 142 viable spores of *Rhizophagus irregularis* per gram of inoculant (www.mykepro.com). Nodulator® peat *Rhizobium* inoculant for pea and lentil (Becker Underwood, Saskatoon, SK) was seed placed across all the treatments at the recommended field rate (5.6 kg ha^{-1}). Field pea (*Pisum sativum* cv. CDC Meadow) was seeded at both locations whereas lentil (*Lens culinaris* cv. CDC Impress) was seeded at Stewart Valley only. A germination test was performed by placing 100 seeds on moist paper towel stored in the dark for 7 d, then determining the number of seeds germinated. The seeds were weighed for a 10 m plot based on the recommended plant density (pea – 85 plants m^{-2}, lentil – 130 plants m^{-2}) (www.agriculture.gov.sk.ca), thousand seed weight (TSW) and germination percentage and seeded through a cone on the seeder at 25 cm row spacing. The MYKE® PRO GR and Nodulator® treatments were applied via the cone with the seed. Canola (*Brassica napus* cv. Clearfield) was seeded perpendicularly adjacent to the experimental plots in both the pea and lentil and served as the non-N_2 fixing reference crop.

The experiment was repeated in 2013 with modifications. A three factor factorial experiment using randomized complete block design was conducted at Stewart Valley, Outlook and Pampbrun. The three factors being three MYKE® PRO GR (Premier Tech, Quebec, Canada) application rates (0, 7.5 kg ha^{-1} and 15 kg ha^{-1}) and two Nodulator® peat *Rhizobium* application rates (0, 1.2 kg per 600 kg seeds) alone and in combination with 16.8 kg P_2O_5 ha^{-1} as mono ammonium phosphate (11-52-0). The total number of plots per rep was 12 with four replicates and individual plot size of 10 × 1.5 m. Field pea was seeded at all the locations whereas lentil was seeded at Stewart Valley only. Seeds were pre-treated with Nodulator® peat *Rhizobium* inoculant on site, and weighed immediately prior to seeding. Inoculant treatments were seeded

after uninoculated treatments. This was done to lessen the possibility of cross-contamination among the treatments due to the peat-based *Rhizobium* inoculant clinging to the cone and seeder. The MYKE® PRO GR treatments were applied via the cone with the seed. Plants were harvested by hand at mid-pod fill stage from a 1-m row length for mid-season above ground biomass and root sampling. The root sampling was performed by excavating the root systems of five plants to a depth of approximately 25 cm with a flat nosed shovel in the harvested rows of each plot. Excess soil was carefully removed and the whole system was transported to the laboratory in plastic bags for washing and analysis. Three canola plants growing closest to the plot were harvested per plot as the reference plant for ^{15}N natural abundance analysis.

At physiological maturity, a hand harvest of three 1-m long rows occurred. Physiological maturity was indicated by yellow upper pods for field pea and tan pods that rattled when shaken for lentil (Saskatchewan Pulse Growers, 2000). A small plot combine was used to harvest the plots at the end of the season during harvest.

3.2. Soil and Plant Analysis

3.2.1. Pre-seeding and post-harvest MPN assay

The most probable number (MPN) method as described by Porter (1979) was modified and performed to estimate the mycorrhizal infective propagules in the soil, prior to seeding and post-harvest. Soil samples to be analysed were bulked among replicates for the post-harvest assay while for pre-seeding, one assay per site was conducted. Diluent soil collected from respective sites was autoclaved for two 30-min (115°C - 138°C) cycles with an interval of 48 h between each cycle and used for the serial dilutions. A series of ten-fold dilutions were carried up to 10^{-5} from the sampled soil. Five replicates were prepared for each dilution level; diluted soils were transferred to Ray Leach "Cone-tainers"™ (Stuewe and Sons Inc., Oregon, USA) and pre-germinated pea seedlings were planted. Seedlings were grown for 36 d in a growth chamber under ambient day/night temperature of 24°C/18°C in a 16 h day. The seedlings were supplied with modified P-free Hoagland and Arnon solution (1950). Plant roots were harvested and washed with deionized H_2O, blotted dry and cut into 1-cm pieces. Cut roots were transferred into a root cassette (VWR Int., Mississauga, ON) lined with cheese cloth, and cleared in 10% potassium hydroxide solution for 15 min, stained in boiling 2% Sheaffer ink-vinegar stain

solution for 3 min (modified from Vierheilig et al., 1998). Root samples were spread on a Petri dish marked with 1-cm gridlines, and observed under a light microscope at 100 X magnification (Olympus SZ61) for the presence or absence of colonization. The most probable number of infective AMF propagules per 100 g of sampled soil was calculated from an MPN table (Cochran, 1950).

3.2.2. Biomass and seeds

Above ground biomass sampled at mid-season and physiological maturity was dried in an indoor facility in cloth bags at 60°C for 72 h. Hand harvested samples were used to determine nutrient uptake in seed and biomass. Samples were stored indoors until further processing. Mid-season samples were weighed prior to grinding. Final harvest samples were weighed and threshed. Total mid-season and final harvest biomass was determined by assessing the dry-weight of three 1-m row samples and multiplied by the row spacing. The seeds were cleaned, weighed and ground for further analyses, and the seed weight was subtracted from the total biomass of the sample to determine the vegetative biomass. Threshed biomass was sub-sampled and ground for straw nutrient analysis. All biomass samples were milled to a < 2-mm particle size in a Wiley mill (Arthur H. Thomas Company, PhiIadelphia, PA). Additionally, plots were harvested using a small plot combine and final seed yields are based on these samples.

Total N and P in all biomass and seed samples were determined by acid-peroxide digestion method (Thomas et al., 1967). According to the methodology, 0.25 g of sample was weighed into 75 mL digestion tubes with 5 mL of concentrated H_2SO_4 and heated at 360°C for 30 min and then allowed to cool. After cooling, 5 mL of H_2O_2 was added to the suspension and heated again. This was repeated five times. The N and P in the solution were measured using a Technicon Autoanalyzer II segmented flow automated colorimetry system (Technicon Instruments Corp., Tarrytown, NY). Total above ground N and P uptake (kg ha^{-1}) during mid-season and harvest, and total seed N and P uptake were calculated by multiplying the determined N and P concentrations by mid-season and final harvest biomass yield, and seed yield, respectively.

3.2.3. Roots

The sampled root systems were gently washed in a solution of 20% (v/v) sodium bicarbonate (modified from Hangs et al., 2012) and water to disaggregate and disperse clays clay lumps, transferred into sieve bags and finally washed in distilled water. Three healthy and whole roots were blotted dry, weighed and cut in quadrants by using steel rulers to prevent bias. Sub-samples were taken from each quadrant to form separate representative sub-samples to evaluate AMF colonization, number of nodules and moisture content. A representative sub-sample was stored at -80℃ for further molecular analysis. For moisture content, the representative sub-sample was dried overnight in an oven at 60°C.

For evaluating nodulation, the number of pink nodules was counted in the representative sub-sample (approx. $1/4^{th}$ of the total root sample). The observed number was multiplied with four (total number of quadrants) to get the total number of nodules per sample and then divided by three (total number of plants evaluated per root sample) to get the final number of nodules per plant. Evaluation of percentage of AMF colonization was performed by modifying the methodology described by Vierheilig et al. (1998). The representative sample was weighed prior to being transferred into a biological sampling cassette (VWR Int., Mississauga, ON) lined with cheese cloth, and cleared by boiling in 10% potassium hydroxide solution for 15 min. The cassettes were rinsed five times thoroughly in tap water to get rid of residual potassium hydroxide and placed in boiling 2% Sheaffer ink-vinegar stain solution for 3 min. The cassettes were rinsed five times in tap water and destained by placing in a solution of tap water and a few drops of vinegar solution for 12 h. Root cassettes were placed in tap water and stored at 4˚C until analysis. A modified gridline intersect method (Giovannetti and Mosse, 1980) was used to evaluate percent AMF colonization. Stained samples were evenly spread with tweezers on a Petri dish marked with 1 cm gridlines, and observed under a light microscope (Olympus SZ61) at 100 X magnification. Gridlines were assessed vertically and horizontally at intersecting root segments for infection. Presence of hyphae, vesicles, arbuscules, or appressoria was recorded at each point where the roots intersected a gridline. The root segments were re-spread and examined four times. The percent colonization was calculated as total number of positive observations out of 100 observations. The percent moisture content of the roots, weight of the

root sub-samples and fresh weight of the total root samples were used to give an estimate of the percent of colonization present in the entire root sample.

For calculating the percent root length colonized, the total root length of the representative sub-sample was calculated by using Newman's equation for total root length:

$$R = \frac{(\pi \text{ N A})}{(2H)} \qquad \text{[Eq. 3.1]}$$

where R = root length of the representative sample (cm); N = total number of intersections occurring; A = area of the Petri dish; and H = total length of grid lines (Newman, 1966).

3.2.4. Biological nitrogen fixation

Mid-season biomass samples were milled to a <2-mm particle size in a Wiley mill (Arthur H. Thomas Company, PhiIadelphia, PA). Subsamples of ground materials were further finely ground in a rotating ball-bearing mill. Approximately 1 mg subsamples were analyzed for total N and atom percent ^{15}N excess with an isotope ratio mass spectrometer VG Micromass 602E (Isotech, Middlewich, England) (Bremer and van Kessel, 1990).

Natural ^{15}N abundance was calculated according to Bremer and van Kessel (1990):

$$\delta\,^{15}N = \left[\frac{atom\%\,^{15}N(sample) - atom\%\,^{15}N(standard)}{atom\%\,^{15}N(standard)}\right] \times 1000 \qquad \text{[Eq. 3.2]}$$

where the standard is atmospheric ^{15}N (0.3663 atom % ^{15}N)

The percent N derived from the atmosphere (%Ndfa) was then calculated as follows:

$$\%Ndfa = \left[\frac{(x-y)}{(x-c)}\right] \times 100 \qquad \text{[Eq. 3.3]}$$

where x is $\delta^{15}N$ of biomass of plants deriving all their N from soil (canola), y is $\delta^{15}N$ of biomass of N-fixing crops (field pea and lentil), and c is $\delta^{15}N$ of biomass of pea and lentil grown in an N-free medium. Values for $\delta^{15}N$ for pea and lentil shoots grown in N-free sand culture were taken from the literature and were -0.66 and -0.56 respectively (Unkovich et al., 2000).

3.3. Statistical Analysis

Statistical analysis was performed using IBM® SPSS© Statistics (Version 20). Results were checked for normality and homogeniety (Levene's test; $p \leq 0.05$ and Shapiro-Wilk test; $p \leq 0.05$). Transformations were performed where the normality and homogeneity were violated. ANOVA was used to assess the significance of AMF, *Rhizobium* and P application. Mean comparisons were performed using Tukey's HSD (Honestly Significant Difference) where ANOVA indicated significance at $p \leq 0.05$. The effect of the treatments was tested through orthogonal contrasts. The planned contrasts tested the effect of recommended rate and twice the recommended rate of AMF (1X and 2X) versus the control (0 AMF). The error terms for all the assessed parameters were homogenous for each year, according to Levene's test for homogeneity. Consequently, data from all sites were combined within years. A mixed linear model was used to assess the effects of site × treatments interactions; treatment was considered as a fixed factor while site was considered as random. The interaction effect error mean squares (MS) were considerably smaller than the main effect error MS, hence it was interpreted that treatment differences were consistent and co-directionally patterned across the sites. According to Mead et al. (2003) and Hinkelmann and Kempthorne (2012) if the ratio of main effect MS error terms and interaction MS error terms are between 1/3 and 1/10 and the homogeneity of error variances among pooled MS error terms of sites is not violated, then a combined site analysis is valid and reasonable compared to separate site analysis.

3.4. Molecular Analyses

3.4.1. Extraction of DNA and pyrosequencing

Roots were subsampled (0.50 g) from each replicate and replicate subsamples were bulked to form a composite sample (2.0 g). Two representative samples weighing approximately

0.75 g each were sub-sampled. Representative root tissue samples were homogenized in 400 µL lysis buffer in 2 mL screw-top micro-centrifuge tubes with three 5-mm ceramic beads by Precellys homogenizer for 3 min. Manufacturer's recommended protocol using a DNeasy® Plant Mini Kit (Qiagen, Maryland, USA) was followed to extract total DNA from the homogenized mixture. The extracted DNA from representative sub-samples were pooled to create the final DNA sample and submitted to Genome Quebec (Montreal, QC) for pyrosequencing analysis.

All the molecular procedures and pyrosequencing were performed on gDNA Genome Quebec Innovation Center. Briefly, the protocol used 5 µL reaction mixtures in first PCR, which consisted of 0.5 µL of 10X buffer, 0.9 µL of 25 mM $MgCl_2$, 0.25 µL of DMSO, 0.1 µL of 10 mM dNTP, 0.05 µL of 5 µM Taq Roche, 1 µL of each primer (NS1 and NS4) in 0.4 µM concentration, 1 µL of diluted DNA (1:10) and 0.2 µL of Ultrapure H_2O. Thermocycler conditions for the first PCR were an initial denaturing step at 95°C for 15 min, 33 cycles of 95°C for 20 s, 50°C for 30 s and 72°C for 90 s, and a final extension step at 72°C for 3 min. The products of the first PCR were purified using Agencourt AMpure XP (Beckman Coulter) following the manufacturer's recommended protocol. The second round of PCR contained 5 µL reaction mixtures of 0.5 µL of 10X buffer, 0.9 µL of 25 mM $MgCl_2$, 0.25 µL of DMSO, 0.1 µL of 10 mM dNTP, 0.05 µL of 5 µM Taq Roche, 1 µL of each primer (AML1-CS1F and AML1-CS1R) in 0.1 µM concentration and 1 µL of undiluted first PCR product. Thermocycler conditions of the second PCR consisted of an initial denaturing step at 95°C for 15 min, 35 cycles of 95°C for 20 s, 60°C for 30 s and 72°C for 45 s, and a final extension step at 72°C for 5 min. A third PCR was conducted to incorporate the bar codes before pooling the samples for sequencing, the 20 µL reaction mixture contained 17 µL of pre-master mix (2 µL of 10X buffer, 3.6 µL of 25 mM $MgCl_2$, 1 µL of DMSO, 0.4 µL of 10 mM dNTP, 0.2 µL of 5 µM Taq Roche and 9.8 µL of Ultrapure H_2O), 2 µL of 454 bar code in 2 µM concentration and 1 µL of undiluted CS1/CS2 PCR product. Thermocycler conditions of the second PCR consisted of an initial denaturing step at 95°C for 10 min, 15 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 1 min, and a final extension step at 72°C for 3 min. The final PCR product was purified using Agencourt AMpure XP (Beckman Coulter) following the manufacturer's recommended protocol and quantified using Picogreen before pooling. Purified samples were tested on Agilent 2100 Bioanalyzer to confirm that PCR products were of the correct size and quality. Final pooled samples were quantified using Qubit® 2.0 Fluorometer from Life Technologies.

3.4.2. Bioinformatics

Mothur version 1.15 (Schloss et al., 2009) was used to process (clean and trim) sequences and generate Operational Taxonomic Units (OTUs). Taxonomic assignment of cleaned sequences was done by alignment and comparison with reference taxa in GenBankOTU by using BLAST. Non-Glomeromycotan sequences were removed from the pool. Representative sequences for each OTU were aligned to construct a phylogenetic tree using the neighbor-joining algorithm in MEGA 5.2.

Sequencing intensity was determined by plotting rarefaction curves for each sample to ensure recovery of maximum AMF sequences and all the curves had either reached or were approaching an asymptote. The diversity indices (richness, Shannon's H, evenness, and phylogenetic diversity) and relative distribution for each genus were also calculated according to Bainard et al. (2014).

4. RESULTS

The early spring in 2012 was climatically cooler and wetter compared to the conditions of a typical growing season, which caused delayed emergence and poor seedling establishment in crops (Section 3, Table 3.2). In 2012, the plots in Kelvington were affected by the presence of Canadian thistle during seedling establishment.

Analysis of homogeneity of variance of pooled error means across the sites is provided in Table 4.1 while the analysis of site, treatment and site × treatments interaction terms is presented in Table 4.2.

4.1 Effects of Inoculation on AMF Colonization and Nodulation

There were no significant effects of any treatment on the percent root length colonized by AMF in either crop in 2012 and 2013. Uninoculated controls were both well nodulated and colonized by the indigenous AMF and *Rhizobium* (Fig. 4.1, 4.2 and 4.3, Table 4.3 and Appendix 8.1). In 2012, the control treatment (0X AMF, 0 P) showed the lowest percent colonization in pea and lentil; in pea, AMF colonization tended to increase with application of AMF at the recommended rate, whereas P had variable effects but the differences were not statistically different (Fig. 4.1). Phosphorus fertilizer application had a significant impact on the number of nodules observed in both pea and lentil. Application of AMF at the recommended rate combined with P produced significant increases in the number of the nodules per plant for pea and lentil, compared to the 0 P treatment or any other AMF treatments applied with or without P. Although nodule numbers in pea were enhanced by the 2X AMF relative to the control, both with and without P, a significant decrease in nodulation of pea relative to the 1X treatment was observed for 2X AMF treatment without P (Fig 4.1).

In 2013, significant interactions were detected between AMF inoculation and P fertilizer, and among AMF, P and *Rhizobium* (Table 4.3), which influenced the number of nodules observed in both pea (Fig. 4.2) and lentil (Fig. 4.3). Application of AMF at the recommended rate combined with P produced a significant increase in the number of nodules per plant for pea and lentil, compared to the 0 P treatment as well as other AMF treatments when applied with or without P. Overall, application of AMF, *Rhizobium* and P had no effect on percent AMF

colonization in pea or lentil in 2013. In 2012, the number of nodules differed between the controls

Table 4.1. Analysis of homogeneity of variance in error terms of assessed parameters (combined between sites) for field pea† in 2012 and 2013.

Treatments	Levene's Test for Homogeneity of Variance for Field pea			
	2012 Field Season		2013 Field Season	
	F‡	*p*‡	F	*p*
AMF colonization (%)	0.3	0.831	0.4	0.797
Number of nodules per plant	0.6	0.490	0.8	0.328
Mid-Biomass	1.3	0.103	0.7	0.261
Biomass P uptake	1.1	0.021	0.8	0.115
Biomass N uptake	1.5	0.326	1.2	0.721
Seed yield§	-	-	0.9	0.883
Seed N uptake§	1.3	0.137	0.7	0.444
Seed P uptake§	1.5	0.211	1.2	0.833
Ndfa (%)	2.3	0.441	1.8	0.211
N fixed	1.4	0.560	0.7	0.108

† Only field pea had multiple sites in 2012 and 2013.

‡ Degrees of freedom and mean squared error terms.

§ Small plot combine seed yield data was available from only Stewart Valley in 2012

Table 4.2. Analysis of site and treatment interactions in assessed parameters (combined between sites) for field pea in 2012 and 2013.

Treatments	Field Pea†			
	2012 Field Season		2013 Field Season	
	df‡	MS‡	df	MS
AMF colonization (%)				
Sites	1	93.33	2	132.17
Treatments	5	69.27	11	101.42
Site × treatments§	5	15.66	22	32.61
Number of nodules per plant				
Sites	1	115.63	2	326.42
Treatments	5	84.52	11	142.30
Site × treatments	5	7.35*	22	15.76*
Mid-Biomass				
Sites	1	384167.82	2	7197196.04
Treatments	5	136139.36	11	65098.46
Site × treatments	5	41051.33	22	11629.00
Final Biomass¶				
Sites	-	-	2	334229.72
Treatments	-	-	11	64167.34
Site × treatments	-	-	22	1072.59
Biomass P uptake				
Sites	1	91.47	2	545.92
Treatments	5	68.09	11	178.30
Site × treatments	5	13.87*	22	55.03*
Biomass N uptake				
Sites	1	112.36	2	461.39
Treatments	5	82.94	11	210.47
Site × treatments	5	21.03*	22	72.06*
Seed yield#				
Sites	-	-	2	3623591.91
Treatments	-	-	11	1001832.90
Site × treatments	-	-	22	109325.60
Ndfa (%)				
Sites	1	532.78	2	708.56
Treatments	5	347.01	11	482.00
Site × treatments	5	107.34	22	187.43
N fixed				
Sites	1	217.03	2	277.44
Treatments	5	154.80	11	102.29
Site × treatments	5	34.22*	22	21.58*

† Only field pea had multiple sites in 2012 and 2013.

‡ Degrees of freedom and mean squared error terms.

§ Site and treatment interaction term.

¶ Final biomass data was available for only one site in 2012.

Small plot combine harvest data for field pea was available for only one site in 2012.

* Mean Square errors are significant at $p < 0.05$

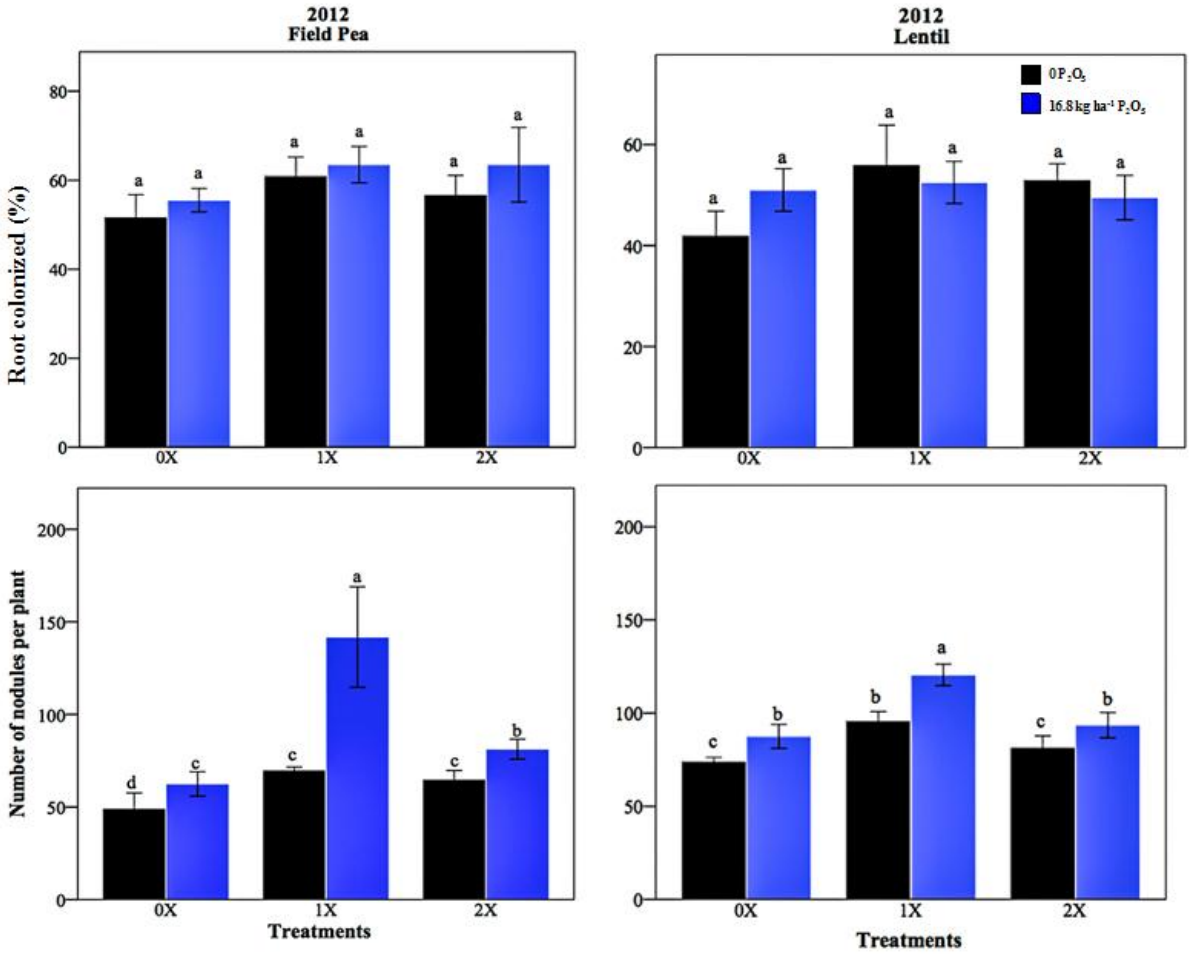


Fig 4.1. Mean percent root length colonized and number of nodules per plant of field pea and lentil in 2012 (averaged across all sites for field pea and at Stewart Valley for lentil). Three levels of arbuscular mycorrhizal fungi (AMF) treatment, 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5, were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

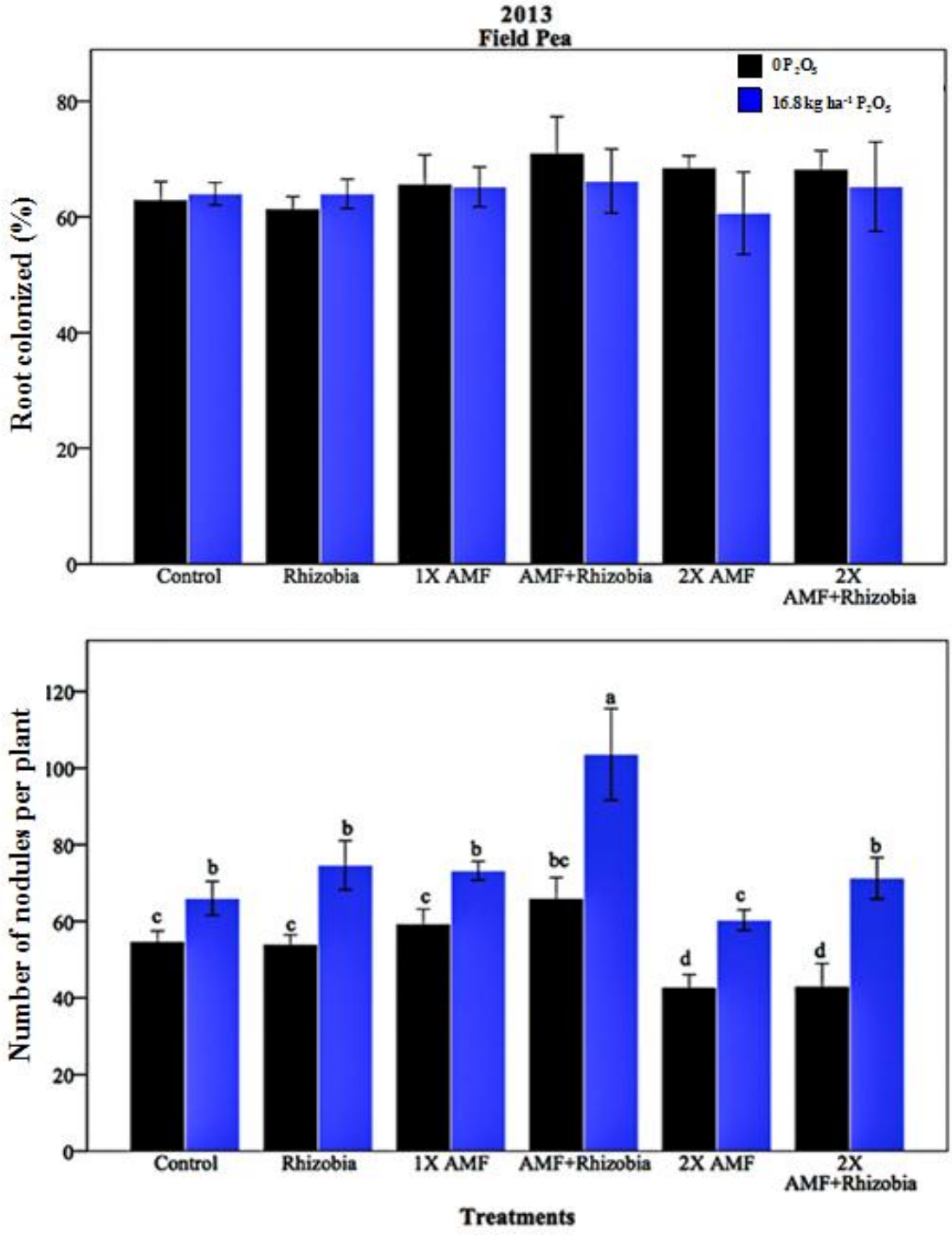


Fig 4.2. Mean % root length colonized and number of nodules per plant of field pea in 2013 (averaged across all sites). Three levels of arbuscular mycorrhizal fungi (AMF) 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

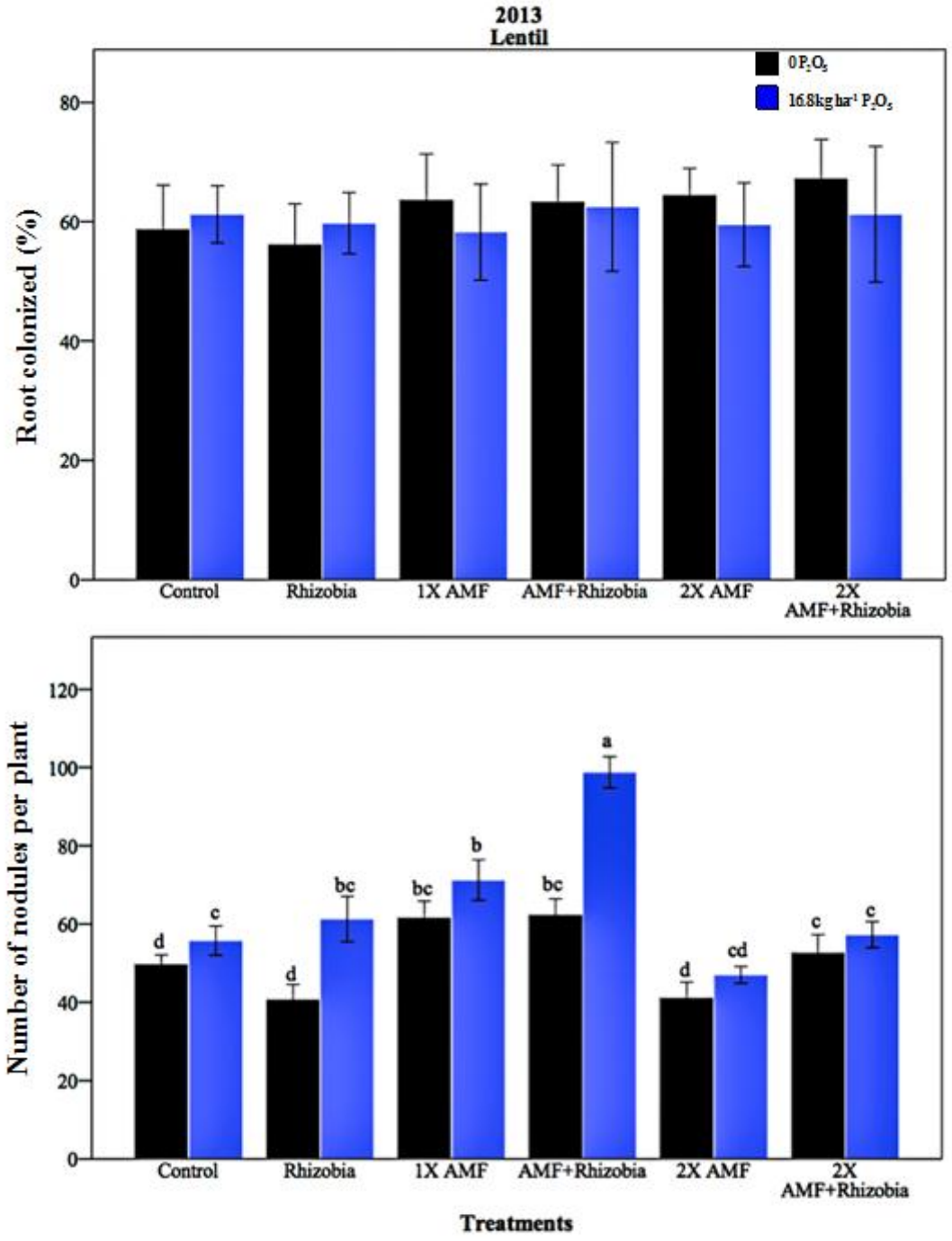


Fig 4.3. Mean % root length colonized and number of nodules per plant of lentil at Stewart Valley in 2013. Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

rate (0 AMF) as compared to AMF inoculation at either the recommended field rate or twice the recommended field rate (1X AMF, 2X AMF) in pea, whereas in lentil the differences were not significant for the same class contrast. In 2013, the class contrast for 0X AMF and 1X AMF was significant in both pea and lentil, while the contrast between the control and AMF application (1X AMF, 2X AMF) was significant only in lentil.

In general, there was an overall increase in nodulation with application of AMF at the recommended rate and a significant increase was observed when AMF was combined with *Rhizobium* and P. An overall significant decrease in nodulation was observed in both the host crops at 2X AMF applications. Root length colonized by AMF was unaffected by the treatments (Fig. 4.1, 4.2 and 4.3). The combined site analysis for the effects of different AMF application rates (0X, 1X and 2X) in combination with *Rhizobium* and P application on percent AMF colonization and nodulation in both the host crops is shown in Table 4.3.

Table 4.3. Combined site analyses of the impact of AMF, P treatments in 2012, and AMF *Rhizobium* and phosphorus treatments in 2013 on AMF colonization and nodulation of field pea and lentil.

Treatments	Field Pea				Lentil			
	AMF Colonization (%)		Nodulation (No. per plant)		AMF Colonization (%)		Nodulation (No. per plant)	
	F†	*p*†	F	*p*	F	*p*	F	*p*
2012 Field Season								
AMF‡	0.4	0.667	0.6	0.355	1.2	0.467	0.3	**0.001**
P§	0.8	0.433	7.2	**0.031**	0.9	0.660	5.7	**0.030**
AMF × P¶	1.1	0.947	5.4	**0.046**	0.3	0.711	4.5	**0.048**
Contrast#								
0X AMF vs 1X, 2X AMF	1.1	0.743	5.1	**0.042**	0.4	0.273	0.1	0.762
0X AMF vs 1X AMF	1.6	0.326	8.1	**0.024**	0.7	0.591	7.9	**0.039**
0X AMF vs 2X AMF	0.2	0.492	1.4	0.091	0.2	0.602	1.2	0.102
2013 Field Season								
AMF	0.7	0.316	0.9	0.372	0.9	0.406	0.3	0.601
P	1.2	0.561	6.5	**0.029**	0.6	0.732	8.1	**0.033**
Rhizobium††	0.6	0.491	1.1	0.093	0.5	0.431	0.5	0.001
AMF × P	0.9	0.799	7.1	**0.048**	0.3	0.666	6.4	**0.043**
AMF × *Rhizobium*‡‡	0.2	0.638	1.8	0.779	0.6	0.491	0.5	0.593
P × *Rhizobium*§§	0.5	0.831	0.5	0.932	0.3	0.642	0.4	0.743
AMF × P × *Rhizobium*¶¶	1.3	0.663	5.2	**0.041**	1.1	0.605	3.4	**0.046**
Contrast								
0X AMF vs 1X, 2X AMF	1.1	0.451	2.1	0.074	0.7	0.520	2.8	**0.049**
0X AMF vs 1X AMF	0.5	0.691	6.1	**0.033**	0.8	0.118	6.4	**0.034**
0X AMF vs 2X AMF	0.1	0.861	1.8	0.083	0.4	0.782	1.8	0.059

† F and *p* values for treatment effects and interaction terms and orthogonal comparison derived from an ANOVA ($p<0.05$).

‡ Three levels of AMF treatment are 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.

§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.

¶ AMF and P interactions.

Orthogonal class contrast = Class comparison of AMF application rates (7.5 kg ha^{-1} and 15 kg ha^{-1}, or average of 7.5 kg ha^{-1} and 15 kg ha^{-1}) as a class vs. none applied.

†† In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, control and recommended rate were used.

‡‡ AMF and *Rhizobium* interactions.

§§ P and *Rhizobium* interactions.

¶¶ AMF, P and *Rhizobium* interactions.

4.2. Effects of Inoculation on Crop Biomass and Nutrient Uptake

4.2.1. Mid-season and harvest biomass

In 2012, no site differences were detected and mid-season biomass means of field pea when combined between Stewart Valley and Kelvington were not significantly (p>0.05) affected by any applied treatments (Fig. 4.4). There was only one site where lentil was sown into the plots in 2012. Overall, a non-significant increase in biomass was observed compared to the control, when AMF was applied at the recommended rate and a decrease was also noted upon application of AMF at 2X. In 2013, there was a significant effect of P fertilizer application on mid-season biomass for both pea and lentil (Fig. 4.5). Significant interaction effects were also observed between AMF inoculation and P fertilizer application in lentil, and among AMF inoculation, P application and *Rhizobium* in field pea. Application of AMF at the recommended rate with P and *Rhizobium* caused an increase in biomass at both mid-season and harvest in both host crops. A significant decrease in biomass was also observed at 2X AMF application rate with and without the combination of P and *Rhizobium*.

The final biomass could be sampled from only Kelvington field pea crop site in 2012 as the plots were accidentally mowed at Stewart Valley. There were no significant differences among the treatments in final biomass for both field pea and lentil at Kelvington and Stewart Valley respectively. Combined site ANOVA is presented in Table 4.2 and the data is provided in Appendix 8.1.

Arbuscular mycorrhizal fungi application rates showed no significant class contrast effects in 2012 in pea as well as lentil for mid-season biomass (Table 4.4). Final harvest biomass results for lentil also showed no significant treatment or AMF class contrast effects. In 2013, application rates of AMF did not show significant class contrast effects in pea and in most of the cases in lentil except between 0X AMF and 2X AMF. Final harvest biomass did not show any significant differences between the treatments and class contrasts in both the host crops.

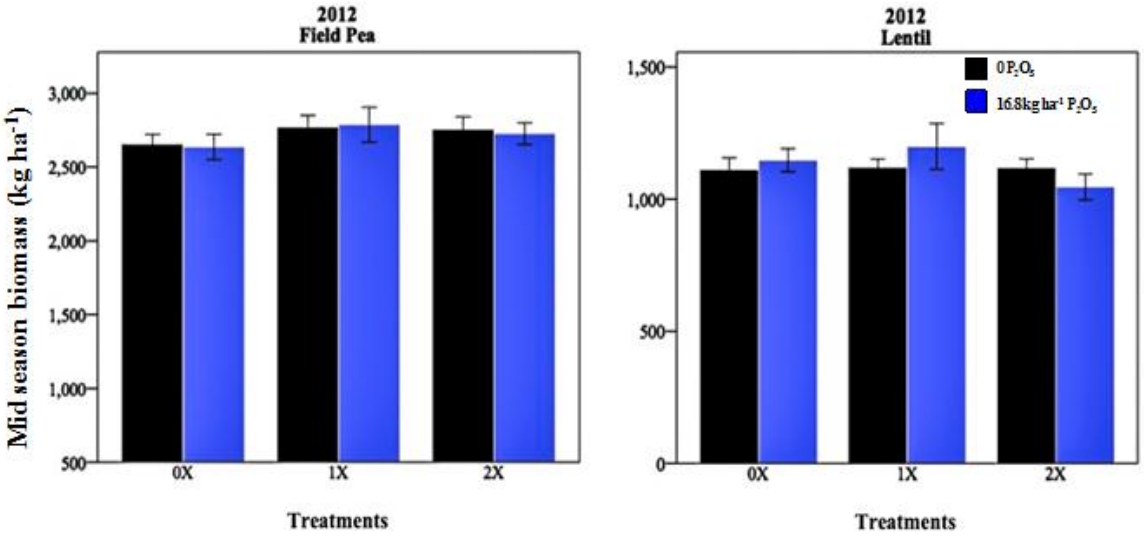


Fig 4.4. Mean mid-season biomass in field pea and lentil in kg ha^{-1} in 2012 (averaged across all sites for field pea and at Stewart Valley for lentil). Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

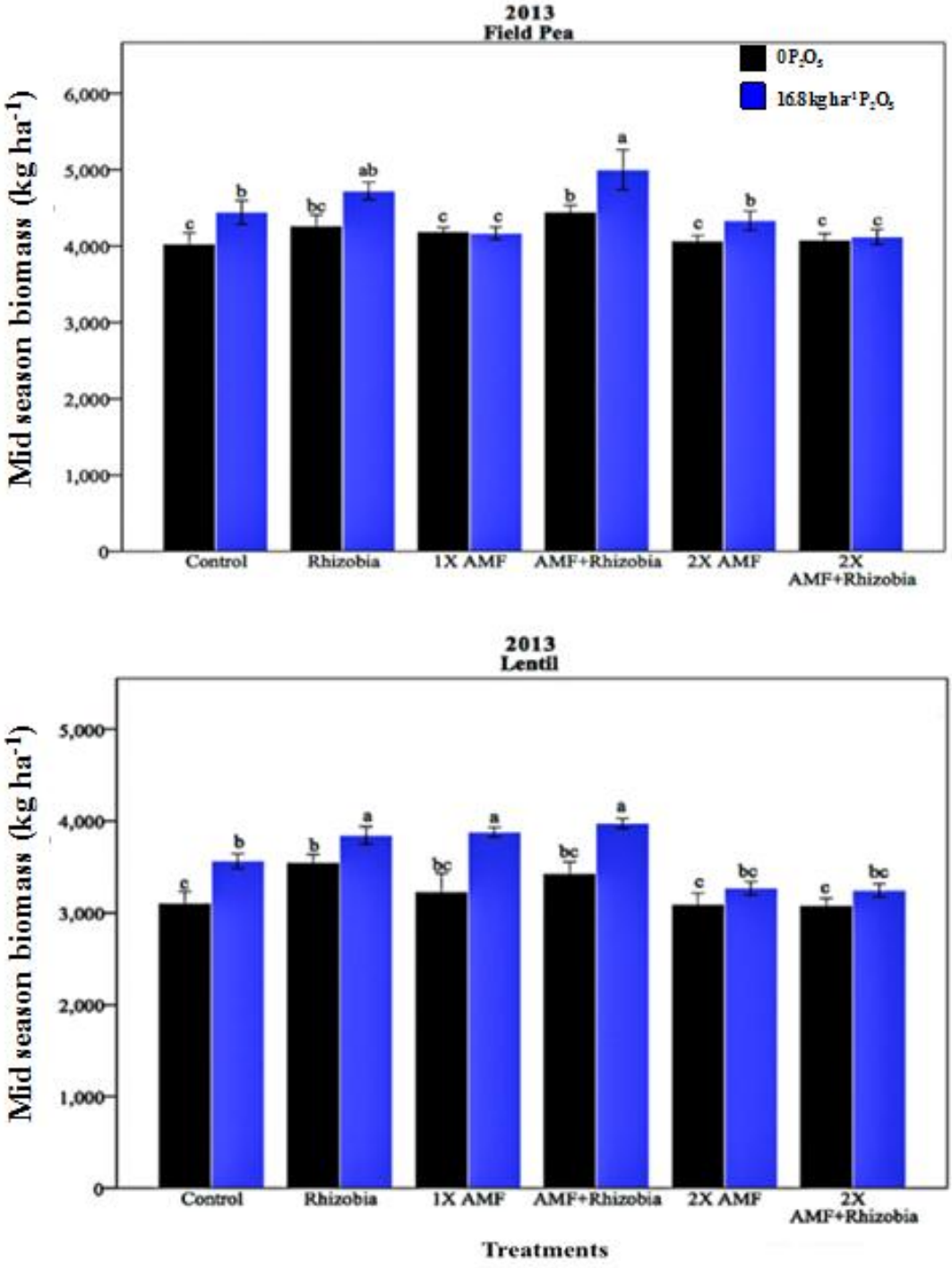


Fig 4.5. Mean mid-season biomass in kg ha^{-1} in field pea and lentil in 2013 (averaged across all sites for field pea and at Stewart Valley for lentil). Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

Table 4.4. Combined site analyses of the impact of AMF, P treatments in 2012, and AMF *Rhizobium* and phosphorus treatments in 2013 on mid-season and final biomass of field pea and lentil.

Treatments	Field Pea				Lentil			
	Mid-season Biomass		Final Biomass†		Mid-season Biomass		Final Biomass	
	F‡	*p*‡	F	*p*	F	*P*	F	*p*
2012 Field Season								
AMF§	0.8	0.621	0.3	0.394	0.4	0.703	0.9	0.344
P¶	1.3	0.389	0.6	0.629	1.8	0.403	1.1	0.563
AMF × P#	0.7	0.543	1.2	0.459	1.8	0.333	0.8	0.290
Contrast††								
0X AMF vs 1X, 2X AMF	0.7	0.721	0.5	0.881	0.4	0.742	0.6	0.496
0X AMF vs 1X AMF	0.5	0.438	0.9	0.795	0.1	0.206	0.9	0.781
0X AMF vs 2X AMF	0.2	0.661	0.7	0.661	0.2	0.809	1.2	0.332
2013 Field Season								
AMF	0.8	0.641	0.9	0.521	0.4	0.533	0.1	0.609
P	7.3	**0.048**	1.3	0.536	11.3	**0.039**	0.3	0.333
Rhizobium‡‡	0.5	0.672	1.2	0.490	0.9	0.688	0.2	0.472
AMF × P	0.7	0.539	0.9	0.122	7.2	**0.043**	0.8	0.533
AMF × *Rhizobium*§§	1.3	0.212	0.6	0.639	1.3	0.106	0.2	0.789
P × *Rhizobium*¶¶	0.9	0.388	0.2	0.544	0.4	0.559	0.3	0.836
AMF × P × *Rhizobium*##	5.3	**0.046**	2.1	0.238	14.6	**0.046**	0.2	0.249
Contrast								
0X AMF vs 1X, 2X AMF	0.7	0.702	1.8	0.563	0.9	0.333	0.5	0.814
0X AMF vs 1X AMF	0.3	0.891	1.2	0.392	2.1	0.099	0.6	0.742
0X AMF vs 2X AMF	1.8	0.070	0.6	0.731	8.1	**0.048**	0.2	0.290

† Final biomass for field pea is only from Kelvington in 2012.

‡ F and *p* values for treatment effects and interaction terms and orthogonal comparison derived from an ANOVA ($p<0.05$).

§ Three levels of AMF treatment are 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.

¶ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.

AMF and P interactions.

†† Orthogonal class contrast = Class comparison of AMF application rates (7.5 kg ha^{-1} and 15 kg ha^{-1}, or average of 7.5 kg ha^{-1} and 15 kg ha^{-1}) as a class vs. none applied.

‡‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, control and recommended rate were applied.

§§ AMF and *Rhizobium* interactions.

¶¶ P and *Rhizobium* interactions.

AMF, P and *Rhizobium* interactions.

4.2.2. Mid-season and final harvest straw nutrient uptake

Combined site analyses were used to assess treatment differences for mid-season N and P uptake in 2012 for field pea. There was only one site with lentil as a host crop in 2012. Mid-season biomass P and N uptake was affected by interaction between AMF and P (Fig. 4.6). In field pea and lentil, biomass P uptake was significantly enhanced by applying P combined with AMF at the recommended rate, compared to the control (0 AMF, 0 P) and 2X AMF treatments. Doubling the application rate of AMF caused a significant decline in P uptake compared to the uptake at recommended rate. An overall increase in biomass N uptake was observed when AMF and P were applied in field pea compared to the control treatment, whereas a significant increase was observed in lentil (Fig. 4.6).

In 2013, P fertilizer application, and interactions between AMF and P fertilizer had an effect on mid-season N and P uptake for both pea (Fig. 4.7) and lentil (Fig. 4.8). In field pea, treatments with AMF and P, and AMF, P, and *Rhizobium* significantly enhanced P uptake relative to the uninoculated control. In lentil, applying AMF, P fertilizer and *Rhizobium* in combination significantly enhanced P uptake. Application of AMF at the recommended rate in combination with P and *Rhizobium* significantly improved N uptake as compared to the control in both pea and lentil. A significant depression in N and P uptake was observed when 2X AMF was applied with or without P and/or *Rhizobium* as compared to co-inoculation of AMF and *Rhizobium* with P in both the host crops.

Final harvest straw nutrient uptake for pea was assessed only at Kelvington in 2012. Straw N and P uptake was not affected by any of the treatments in lentil in 2012. In 2013, no significant treatment effects were observed for straw N and P uptake in field pea and lentil. A non-significant overall trend of increased P and N uptake compared to the control was observed when a combination of AMF, *Rhizobium* and P was applied in both the host crops. A non-significant depression in nutrient uptake was also observed at higher rates of AMF application (Appendix A.1).

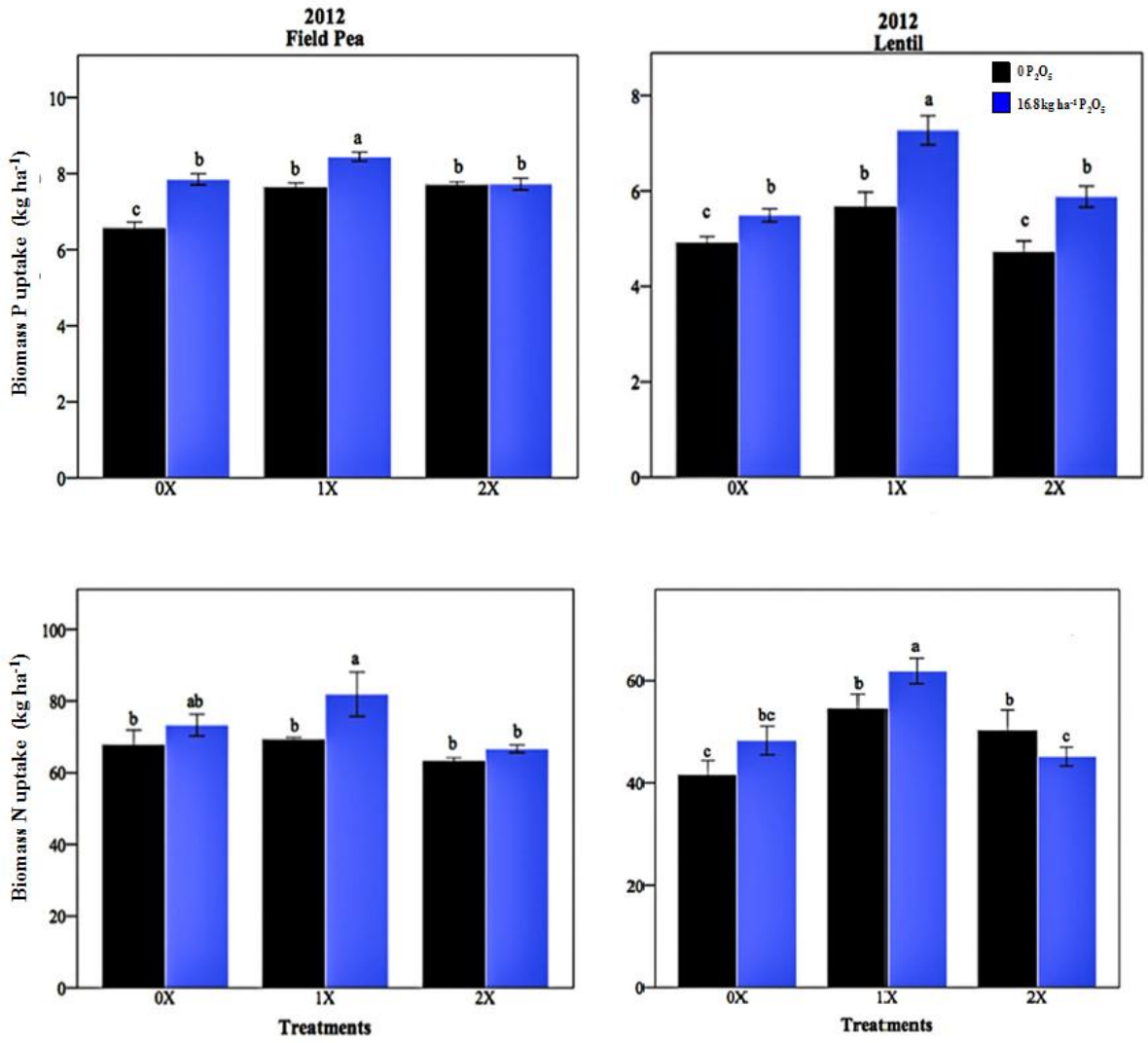


Fig 4.6. Mean mid-season biomass P and N uptake in kg ha^{-1} in field pea and lentil in 2012 (averaged across all sites for field pea and at Stewart Valley for lentil). Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

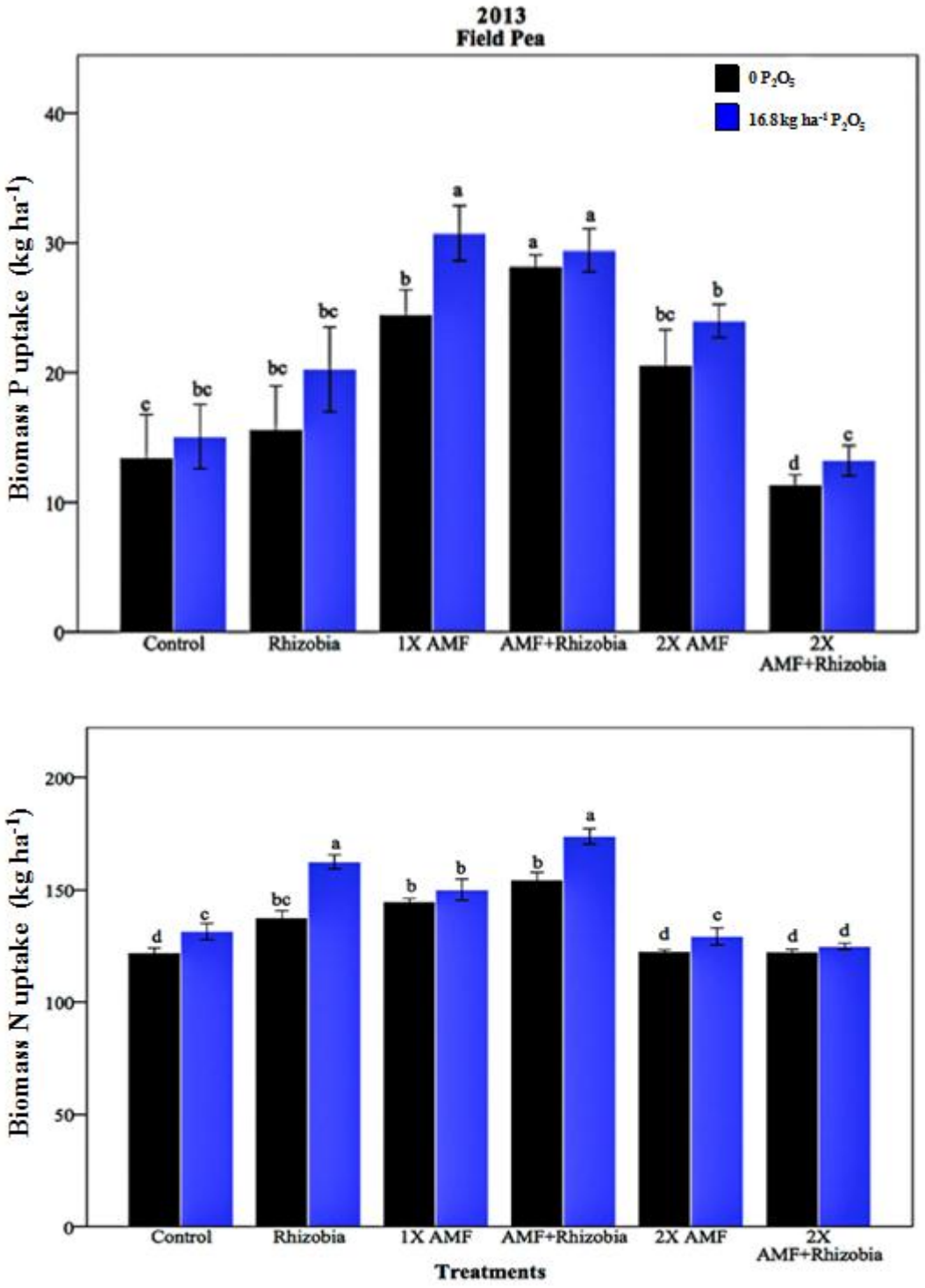


Fig 4.7. Mean mid-season biomass P and N uptake in kg ha^{-1} in field pea in 2013 (averaged across all sites). Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 was applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

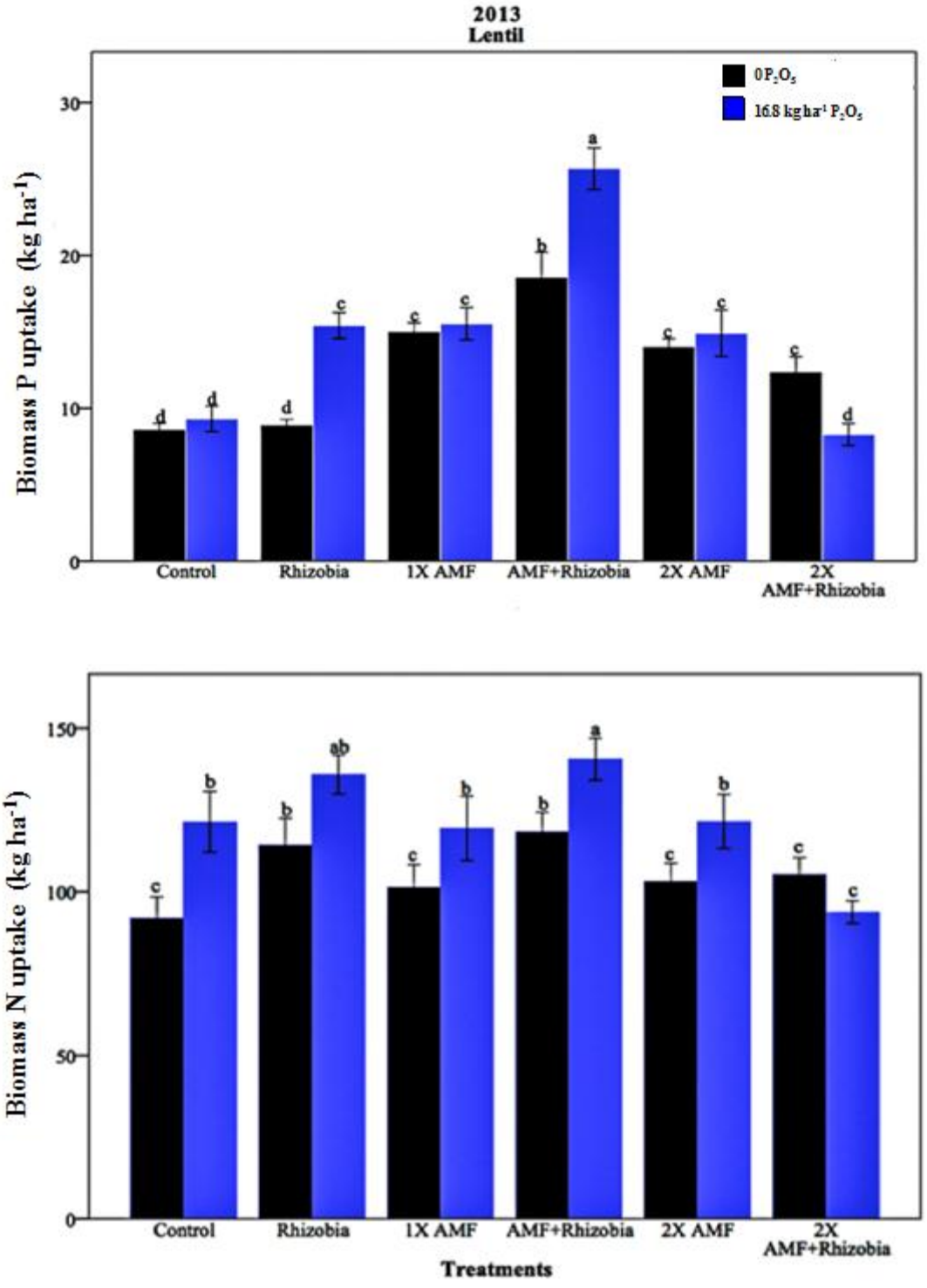


Fig 4.8. Mean mid-season biomass P and N uptake in kg ha^{-1} in lentil at Stewart Valley in 2013. Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 was applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

In 2012, the class contrasts for AMF application rates did not show any significant differences for mid-season N and P uptake for field pea (Table 4.5). For lentil, class contrast effects were observed for both N and P uptake (Table 4.6). Variation effect for P and interaction effects for AMF and P was significant for N and P uptake in both field pea and lentil. Class contrast effects ($p<0.05$) between all the three rates of AMF application were observed in lentil for both N and P uptake. In case of field pea the class contrast was significant only between 0X AMF and 1X AMF for N and P uptake.

Straw N and P uptake showed no significant interaction effects for either field pea or lentil (Table 4.5 and 4.6). Class contrast effects for different rates of AMF application for straw N and P uptake were observed to be non-significant for field pea and variable in lentil.

Table 4.5. Combined site analyses of the impact of AMF, P treatments in 2012, and AMF *Rhizobium* and phosphorus treatments in 2013 on mid season and final biomass N and P uptake of field pea.

Treatments	Mid Biomass				Final Biomass†			
	Total N		Total P		Total N		Total P	
	F‡	*p*‡	F	*p*	F	*P*	F	*p*
2012 Field Season								
AMF§	0.2	0.799	0.4	0.821	0.5	0.88	0.3	0.83
P¶	4.1	**0.050**	5.8	**0.048**	0.2	0.39	0.7	0.55
AMF × P#	3.5	**0.049**	5.1	**0.047**	0.9	0.65	0.9	0.71
Contrast††								
0X AMF vs 1X, 2X AMF	0.8	0.661	1.4	0.084	0.3	0.52	0.8	0.33
0X AMF vs 1X AMF	0.9	0.216	2.8	0.433	0.7	0.91	0.5	0.47
0X AMF vs 2X AMF	1.2	0.138	0.6	0.663	0.4	0.47	0.6	0.81
2013 Field Season								
AMF	0.7	0.591	1.6	0.499	0.4	0.778	0.5	0.461
P	4.9	**0.048**	9.8	**0.033**	1.3	0.639	0.8	0.328
Rhizobium††	0.9	0.695	1.8	0.391	0.2	0.772	0.3	0.671
AMF × P	4.2	**0.044**	1.8	0.159	0.4	0.832	0.6	0.799
AMF × *Rhizobium*‡‡	4.5	**0.041**	5.9	**0.039**	0.9	0.691	0.8	0.631
P × *Rhizobium*§§	0.5	0.566	0.4	0.632	0.1	0.455	0.3	0.852
AMF × P × *Rhizobium*¶¶	7.7	**0.041**	12.1	**0.037**	1.3	0.593	0.9	0.164
Contrast								
0X AMF vs 1X, 2X AMF	0.6	0.344	7.7	**0.044**	0.3	0.891	0.2	0.892
0X AMF vs 1X AMF	6.4	**0.039**	18.9	**0.028**	0.8	0.571	0.3	0.774
0X AMF vs 2X AMF	0.1	0.451	0.8	0.754	6.9	**0.049**	0.7	0.385

† Final biomass was taken only at Kelvington for field pea in 2012.

‡ F and *p* values for treatment effects and interaction terms and orthogonal comparison derived from an ANOVA ($p<0.05$).

§ Three levels of AMF treatment are 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.

¶ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.

AMF and P interactions.

†† Orthogonal class contrast = Class comparison of AMF application rates (7.5 kg ha^{-1} and 15 kg ha^{-1}, or average of 7.5 kg ha^{-1} and 15 kg ha^{-1}) as a class vs. none applied.

‡‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, control and recommended rate were applied.

§§ AMF and *Rhizobium* interactions.

¶¶ P and *Rhizobium* interactions.

AMF, P and *Rhizobium* interactions.

Table 4.6. Combined site analyses of the impact of AMF, P treatments in 2012, and AMF *Rhizobium* and phosphorus treatments in 2013 on mid season and final biomass N and P uptake of lentil.

Treatments	Mid Biomass				Final Biomass†			
	Total N		Total P		Total N		Total P	
	F‡	*p*‡	F	*p*	F	*p*	F	*p*
2012 Field Season								
AMF§	1.2	0.155	0.9	0.109	0.1	0.305	0.1	0.730
P¶	7.1	**0.033**	8.5	**0.032**	0.5	0.449	0.3	0.538
AMF × P#	11.6	**0.034**	12.8	**0.043**	0.6	0.300	0.4	0.526
Contrast††								
0X AMF vs 1X, 2X AMF	6.7	**0.044**	7.3	**0.033**	0.1	0.834	0.1	0.884
0X AMF vs 1X AMF	16.1	**0.004**	6.1	**0.045**	0.1	0.832	0.1	0.733
0X AMF vs 2X AMF	8.2	**0.033**	0.3	0.834	0.3	0.799	0.4	0.672
2013 Field Season								
AMF	0.7	0.180	1.3	0.365	0.1	0.366	0.2	0.730
P	8.5	**0.026**	6.3	**0.042**	0.5	0.589	0.6	0.538
Rhizobium‡‡	1.3	0.193	1.3	0.733	0.2	0.641	0.2	0.390
AMF × P	8.9	**0.033**	5.9	**0.048**	0.9	0.632	0.7	0.733
AMF × *Rhizobium*§§	5.1	**0.046**	6.3	**0.032**	0.4	0.833	0.6	0.893
P × *Rhizobium*¶¶	0.7	0.599	0.5	0.338	0.3	0.752	0.2	0.659
AMF × P × *Rhizobium*##	10.2	**0.039**	14.6	**0.033**	0.7	0.794	0.5	0.641
Contrast								
0X AMF vs 1X, 2X AMF	16.1	**0.004**	21.7	**0.005**	0.7	0.633	0.4	0.741
0X AMF vs 1X AMF	21.1	**0.003**	23.3	**0.003**	0.6	0.698	7.3	**0.049**
0X AMF vs 2X AMF	9.1	**0.023**	9.8	**0.026**	0.6	0.433	0.3	0.559

† Final biomass was taken only at Kelvington for field pea in 2012.

‡ F and *p* values for treatment effects and interaction terms and orthogonal comparison derived from an ANOVA ($p<0.05$).

§ Three levels of AMF treatment are 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.

¶ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.

AMF and P interactions.

†† Orthogonal class contrast = Class comparison of AMF application rates (7.5 kg ha^{-1} and 15 kg ha^{-1}, or average of 7.5 kg ha^{-1} and 15 kg ha^{-1}) as a class vs. none applied.

‡‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, control and recommended rate were applied.

§§ AMF and *Rhizobium* interactions.

¶¶ P and *Rhizobium* interactions.

AMF, P and *Rhizobium* interactions.

4.4. Effects of Inoculation on Seed Yield and Seed Nutrient Uptake

4.4.1. Seed yield

Seed yield for both field pea and lentil was estimated through hand harvest sampling (Appendix A, Fig. A.1, A.2 and Table A.7) as well as small plot combine sampling at crop maturity (Figure 4.9). Hand-harvested samples were based on a relatively small sampling area, and are more likely to be subject to inadvertent sampling bias and thus the seed yield samples based on the small-plot combine samples are considered to be a better representation of the yield. In 2012, no statistically significant differences in seed yield means of field pea and lentil were detected, irrespective of P or AMF application (Fig. 4.9). Although data suggest an overall trend of seed yield increases in both host crops with the application of AMF inoculant in combination with P fertilizer, none of the treatment main effects or simple effects were considered statistically significant (Table 4.7).

In 2013, there was no significant effect of P fertilizer application and interaction between AMF and P on seed yield in field pea and lentil (Fig. 4.10). Application of AMF at the recommended rate in combination with P and *Rhizobium* caused a small enhancement of seed yield compared to the other treatments in lentil; however, the differences in seed yield were not statistically significant. Doubling the rate of AMF application with or without in P and *Rhizobium* appeared to cause a depression in seed yield, although the differences in seed yields were not statistically significant. As was observed in 2012, none of the treatment effects were considered statistically significant.

ANOVA analysis of data combined among the sites for the effects of different application rates of AMF (0X, 1X and 2X) in combination with *Rhizobium* and P fertilizer on seed yield in both the host crops is presented in Table 4.7. Class contrast effects of AMF application rates of 0 AMF vs 1X AMF showed non-significant results in 2012 in field pea and lentil (Table 4.7). Similarly in 2013, class contrasts of application rates of AMF were not significant for field pea and lentil.

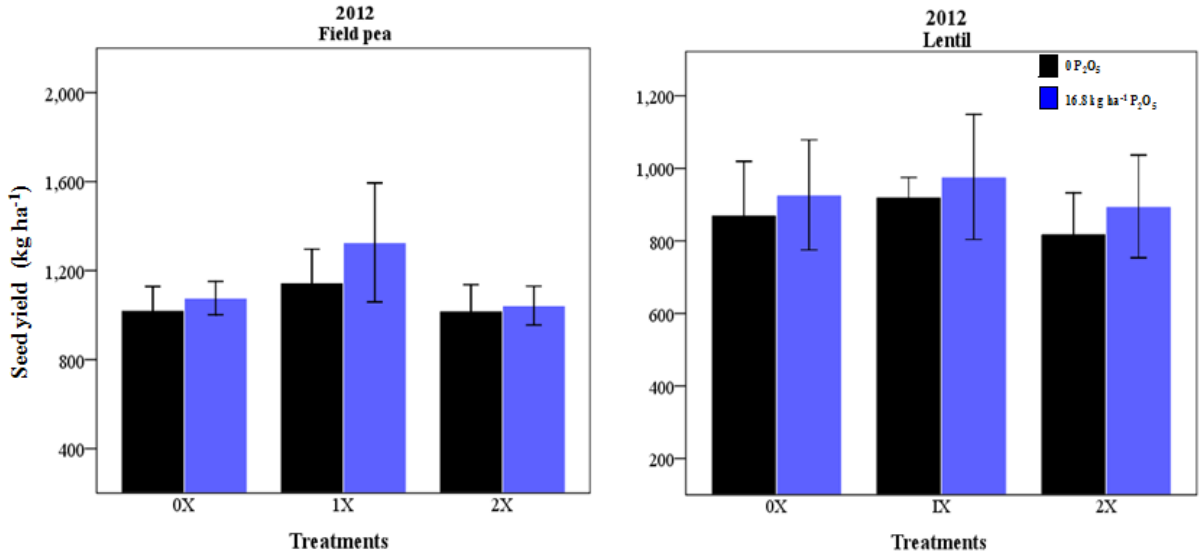


Fig 4.9. Mean seed yield (small plot combine) in kg ha^{-1} in field pea and lentil in 2012 at Stewart Valley. Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

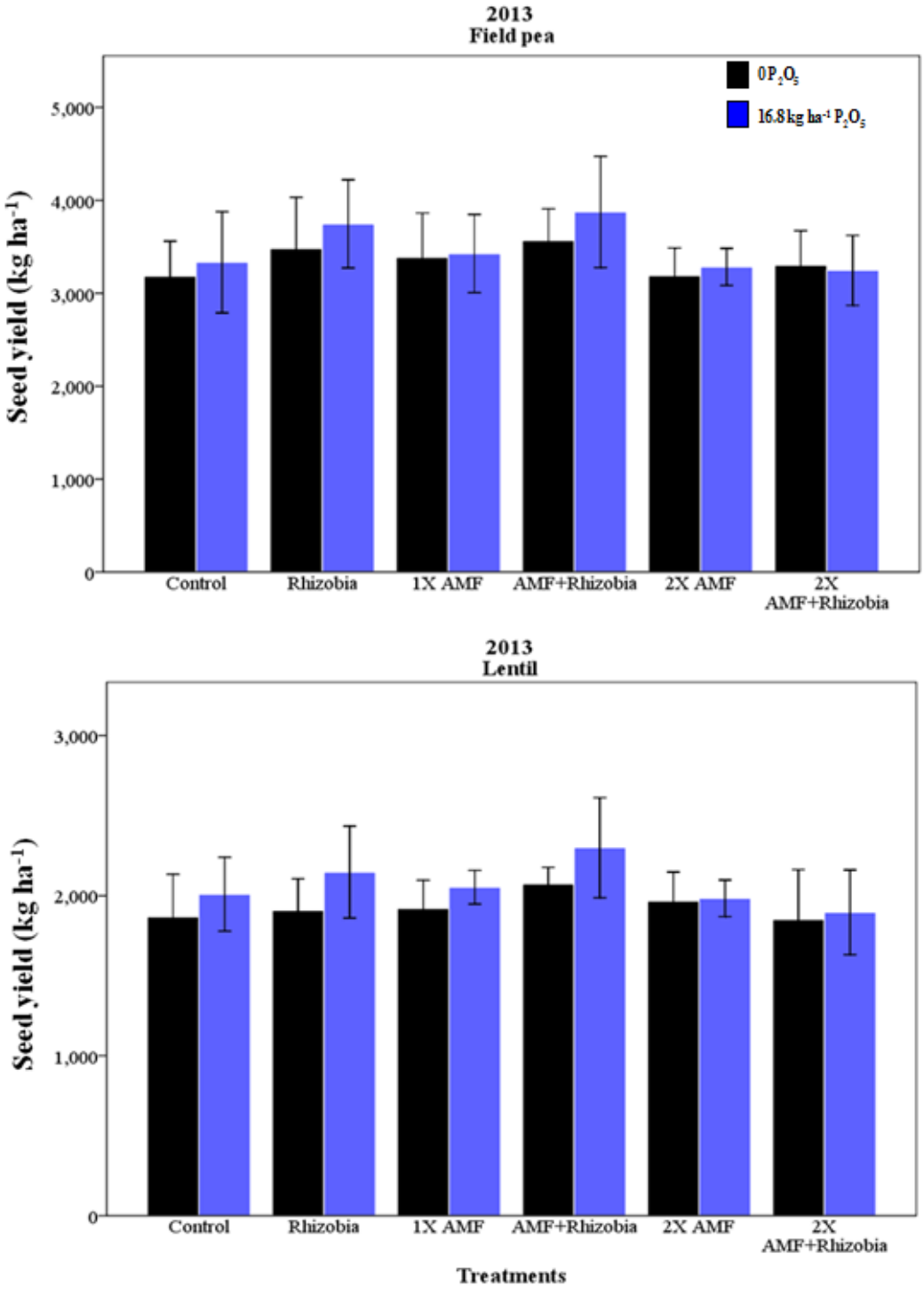


Fig 4.10. Mean seed yield in kg ha^{-1} in field pea and lentil in 2013(averaged across all sites for field pea and at Stewart Valley for lentil). Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 was applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

Table 4.7. Combined site analyses of the impact of AMF, P treatments in 2012, and AMF *Rhizobium* and phosphorus treatments in 2013 seed yield (small plot combine) of field pea and lentil.

Treatments	Field Pea		Lentil	
	Seed Yield†		Seed Yield	
	F‡	*p*‡	F	*p*
2012 Field Season				
AMF§	0.2	0.391	0.6	0.884
P¶	1.3	0.582	0.4	0.739
AMF × P#	0.9	0.731	0.5	0.833
Contrast††				
0X AMF vs 1X, 2X AMF	0.4	0.441	0.9	0.338
0X AMF vs 1X AMF	0.6	0.861	0.7	0.985
0X AMF vs 2X AMF	0.8	0.947	0.6	0.389
2013 Field Season				
AMF	0.7	0.341	0.9	0.487
P	0.9	0.639	0.7	0.453
Rhizobium‡‡	0.8	0.991	0.2	0.367
AMF × P	0.4	0.432	0.9	0.766
AMF × *Rhizobium*§§	0.3	0.877	0.5	0.529
P × *Rhizobium*¶¶	0.8	0.938	0.6	0.662
AMF × P × *Rhizobium*##	0.9	0.544	0.9	0.838
Contrast				
0X AMF vs 1X, 2X AMF	0.6	0.943	0.7	0.453
0X AMF vs 1X AMF	0.8	0.491	0.4	0.671
0X AMF vs 2X AMF	0.9	0.821	0.8	0.837

† Small plot combine yield for field pea was only available at Stewart Valley in 2012.

‡ F and *p* values for treatment effects and interaction terms and orthogonal comparison derived from an ANOVA ($p<0.05$).

§ Three levels of AMF treatment are 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.

¶ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.

AMF and P interactions.

†† Orthogonal class contrast = Class comparison of AMF application rates (7.5 kg ha^{-1} and 15 kg ha^{-1}, or average of 7.5 kg ha^{-1} and 15 kg ha^{-1}) as a class vs. none applied.

‡‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, control and recommended rate were applied.

§§ AMF and *Rhizobium* interactions.

¶¶ P and *Rhizobium* interactions.

AMF, P and *Rhizobium* interactions.

4.4.2. Seed nutrient uptake

Seed nutrient uptake was calculated based on the hand-harvested samples. In 2012 seed N and P uptake were significantly affected by P fertilizer application and a significant interaction between AMF and P fertilizer application was detected for both pea and lentil (Fig. 4.11). Application of P in combination with AMF at the recommended rate significantly enhanced P and N uptake in field pea and lentil, compared to the control (0 AMF, 0 P) and 2X AMF treatments. A significant decline in P and N uptake was observed compared to the uptake at recommended rate in both the host crops at 2X rate of AMF application.

In 2013, P fertilizer application, interactions between AMF and P fertilizer and interaction between AMF, P and *Rhizobium* had a significant effect on seed N and P uptake in both the host crops (Fig. 4.12 and 4.13). Significantly higher P uptake was observed in field pea when P was applied with *Rhizobium* and the combination of AMF and *Rhizobium*, compared to the control as well as other treatments. Seed N uptake increased significantly when *Rhizobium* was applied alone, with P, and combined with AMF and P. Similar results were observed in lentil where P and N uptake in seed was enhanced significantly when *Rhizobium* and P fertilizer was applied alone and with AMF. A significant suppression effect was observed in the uptake of nutrients when AMF was applied at 2X of the recommended rate with and without P and *Rhizobium*, compared to co-inoculation of *Rhizobium* and AMF at the recommended rate with P in both the host crops.

Significant class contrasts for AMF application rates were observed in 2012 for seed N and P uptake in field pea and lentil (Table 4.8). A significant P effect and interaction effects for AMF and P were observed for N and P uptake in both field pea and lentil. The interaction, variation and class contrast effects were variable in 2013. Significant class contrast effects between AMF application rates were observed in field pea for only N uptake. A significant P effect was observed in both the host crops for N and P uptake, while the interaction of P with AMF was significant only in lentil and significant interactions of *Rhizobium* with P were detected only for N uptake in field and lentil. The interaction effect for AMF, P and *Rhizobium* had a significant effect on N and P uptake in field pea as well as lentil.

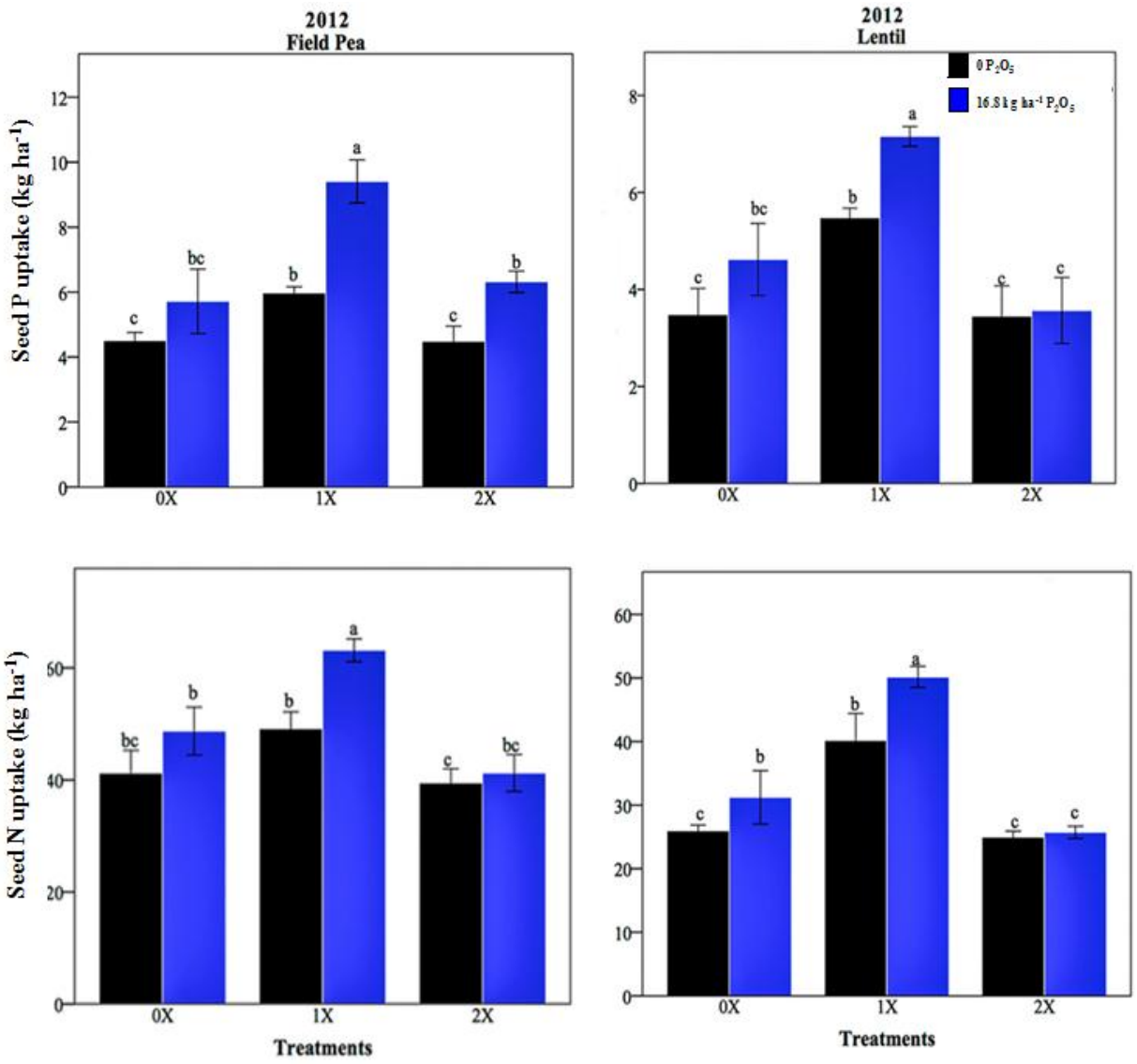


Fig 4.11. Mean seed P and N uptake in kg ha^{-1} in field pea and lentil in 2012 at Stewart Valley. Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

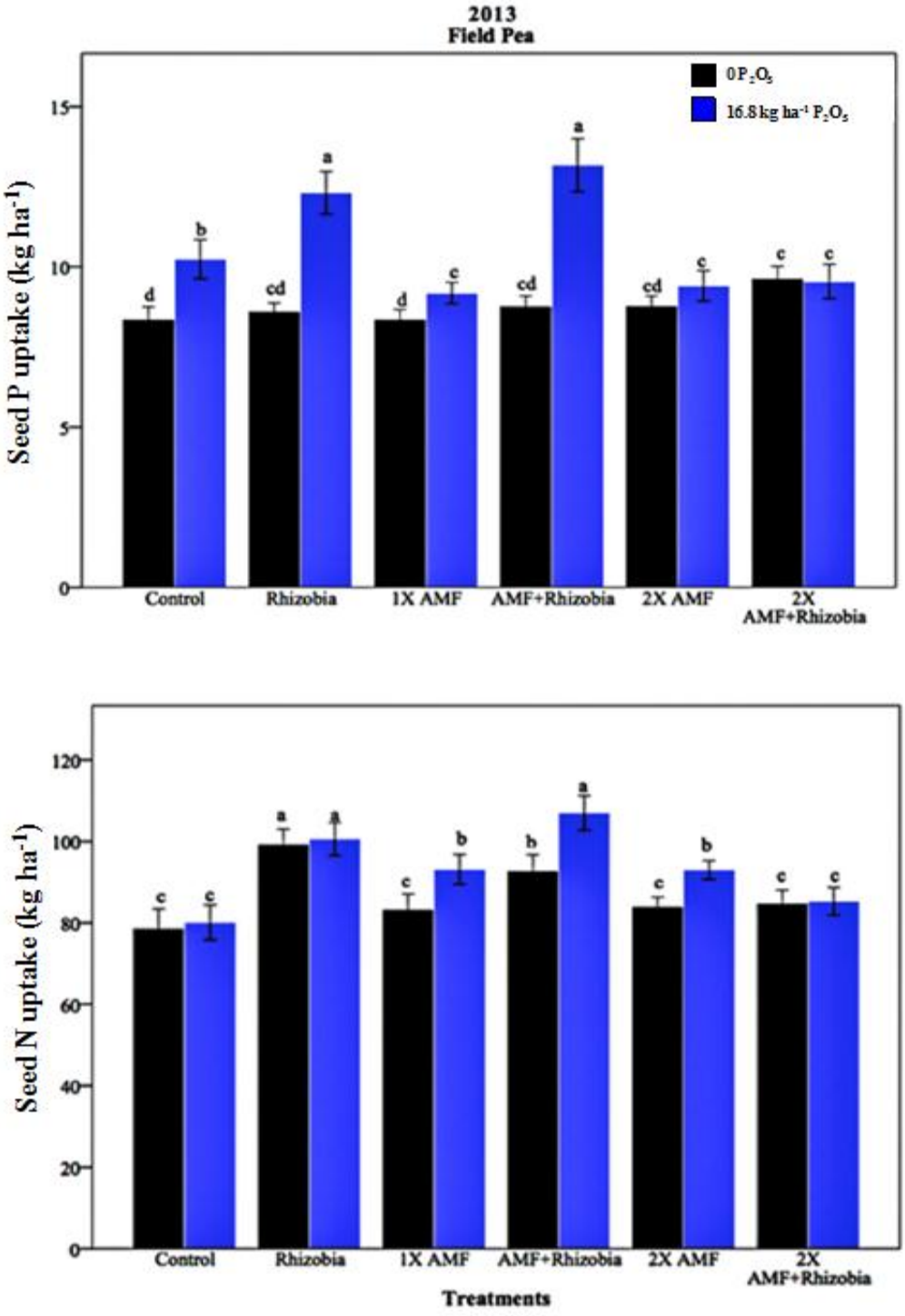


Fig 4.12. Mean seed P and N uptake in kg ha^{-1} in field pea in 2013 (averaged across all sites). Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$)

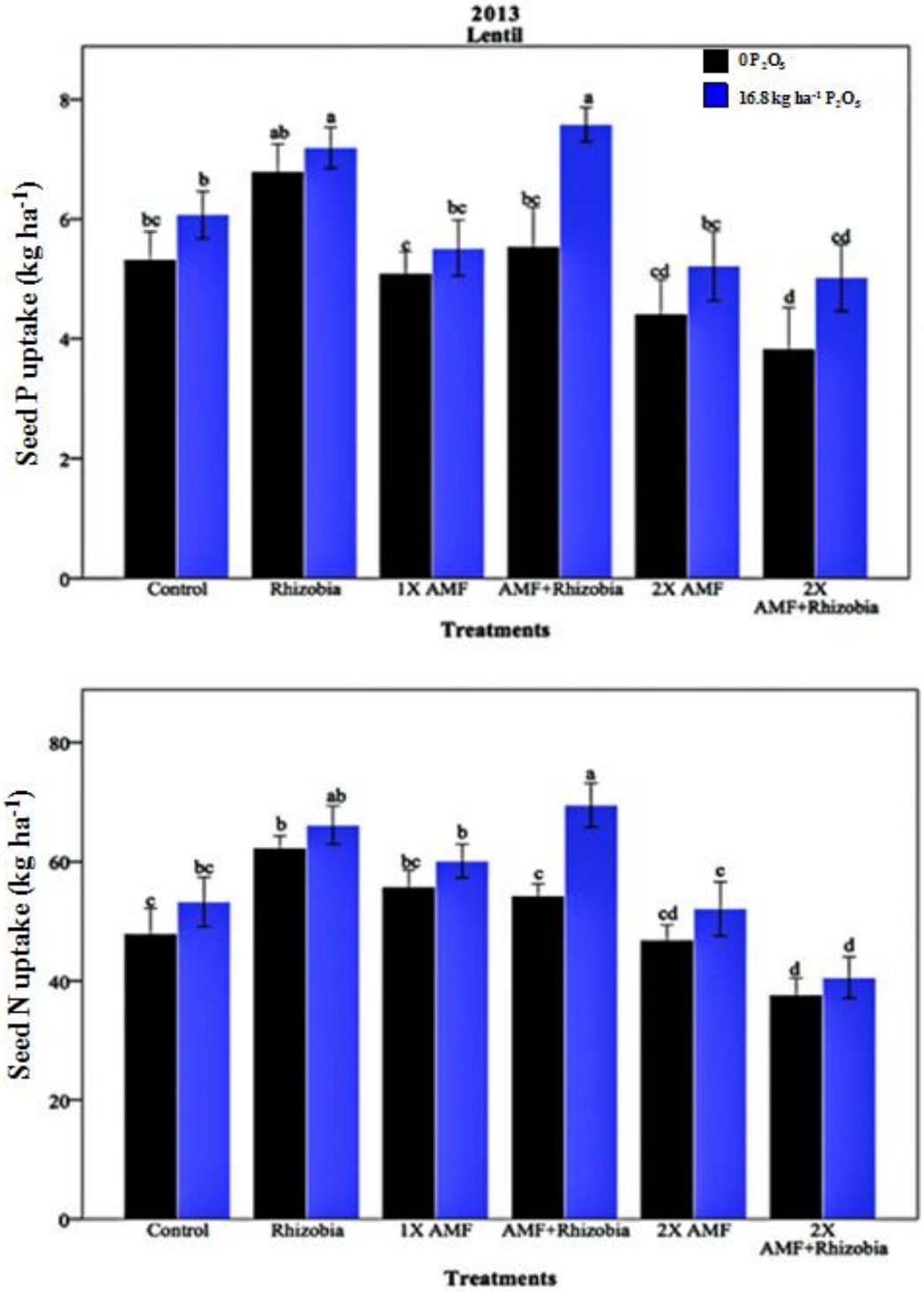


Fig 4.13. Mean seed P and N uptake in kg ha^{-1} in lentil at Stewart Valley in 2013. Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

Table 4.8. Combined site analyses of the impact of AMF, P treatments in 2012, and AMF *Rhizobium* and phosphorus treatments in 2013 seed N and P uptake of field pea and lentil.

Treatments[†]	Field Pea				Lentil			
	Total N[†]		Total P[†]		Total N		Total P	
	F[‡]	*p*[‡]	F	*p*	F	*p*	F	*p*
2012 Field Season								
AMF[§]	0.7	0.285	0.5	0.507	0.5	0.582	0.8	0.383
P[¶]	15.1	**0.023**	7.6	**0.042**	7.5	**0.041**	6.3	**0.033**
AMF × P[#]	8.1	**0.044**	5.2	**0.041**	6.6	**0.044**	4.1	**0.042**
Contrast[††]								
0X AMF vs 1X, 2X AMF	5.7	**0.048**	6.6	**0.043**	4.5	**0.043**	3.1	**0.049**
0X AMF vs 1X AMF	7.2	**0.041**	5.1	**0.047**	13.1	**0.035**	5.4	**0.042**
0X AMF vs 2X AMF	6.2	**0.039**	0.1	0.793	0.5	0.742	0.1	0.834
2013 Field Season								
AMF	0.5	0.670	0.3	0.935	0.5	0.526	0.4	0.495
P	6.1	**0.048**	7.8	**0.047**	5.5	**0.043**	4.9	**0.048**
Rhizobium[‡‡]	1.3	0.193	1.3	0.733	0.5	0.644	0.3	0.792
AMF × P	1.4	0.210	1.3	0.333	4.1	**0.044**	4.7	**0.040**
AMF × *Rhizobium*[§§]	0.9	0.322	0.8	0.583	0.2	0.499	0.8	0.535
P × *Rhizobium*[¶¶]	5.2	**0.042**	1.0	0.648	4.3	**0.033**	1.3	0.663
AMF × P × *Rhizobium*[##]	11.2	**0.038**	5.6	**0.049**	10.3	**0.030**	5.3	**0.037**
Contrast								
0X AMF vs 1X, 2X AMF	5.3	**0.041**	1.7	0.542	0.1	0.331	0.9	0.321
0X AMF vs 1X AMF	11.3	**0.040**	0.3	0.602	1.5	0.109	0.7	0.749
0X AMF vs 2X AMF	0.1	0.366	0.8	0.557	1.2	0.438	0.8	0.548

† Small plot combine yield for field pea was only available at Stewart Valley in 2012.

‡ F and *p* values for treatment effects and interaction terms and orthogonal comparison derived from an ANOVA ($p<0.05$).

§ Three levels of AMF treatment are 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.

¶ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.

AMF and P interactions.

†† Orthogonal class contrast = Class comparison of AMF application rates (7.5 kg ha^{-1} and 15 kg ha^{-1}, or average of 7.5 kg ha^{-1} and 15 kg ha^{-1}) as a class vs. none applied.

‡‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, control and recommended rate were applied.

§§ AMF and *Rhizobium* interactions.

¶¶ P and *Rhizobium* interactions.

AMF, P and *Rhizobium* interactions.

4.5. Effects of Inoculation on Biologically Fixed Nitrogen

For consistency, total biologically fixed N values are based on the hand-harvested samples. In 2012, the percentage of N directly fixed from atmosphere (%Ndfa) and total biologically fixed N was significantly affected by P fertilizer and AMF treatments in field pea and lentil (Fig. 4.14). Application of AMF at the recommended rate with *Rhizobium* and P significantly increased %Ndfa and total biologically fixed N compared to the control and other treatments in field pea and lentil. Increasing the application of AMF to twice the recommended rate caused a significant decline in biological N fixation in the host crops.

In 2013, application of AMF at the recommended rate with P and with *Rhizobium* and P in field pea caused a significant increase in %Ndfa, compared to the control treatment. Application of *Rhizobium* with P also caused a significant enhancement in %Ndfa compared to the control, but it was also significantly lower compared to when AMF was inoculated along with P (Fig. 4.15). Interestingly, the highest increase in total biologically fixed N was observed when AMF was applied at the recommended rate with *Rhizobium* and P, compared to all other treatments. Total biologically fixed N was also significantly enhanced compared to the control when only *Rhizobium* and AMF were applied with P fertilizer. Similar effects were seen in the case of lentil; application of P with AMF at the recommended rate or with *Rhizobium* and co-inoculation of AMF, *Rhizobium* and P significantly enhanced %Ndfa compared to the control (Fig. 4.16). Total biologically fixed N was also significantly enhanced compared to the control when AMF was applied at the recommended rate along with *Rhizobium* and P. Application of only *Rhizobium* or only AMF along with P also caused a similar increase compared to the control, but they were significantly lower compared to the effect of co-inoculation with P. Overall, in 2013 biological N fixation was significantly improved when AMF inoculant was applied in combination with P fertilizer and *Rhizobum* in both pea and lentil. The benefits conferred by such interactions were not observed when the rate of AMF inoculant application was increased two fold.

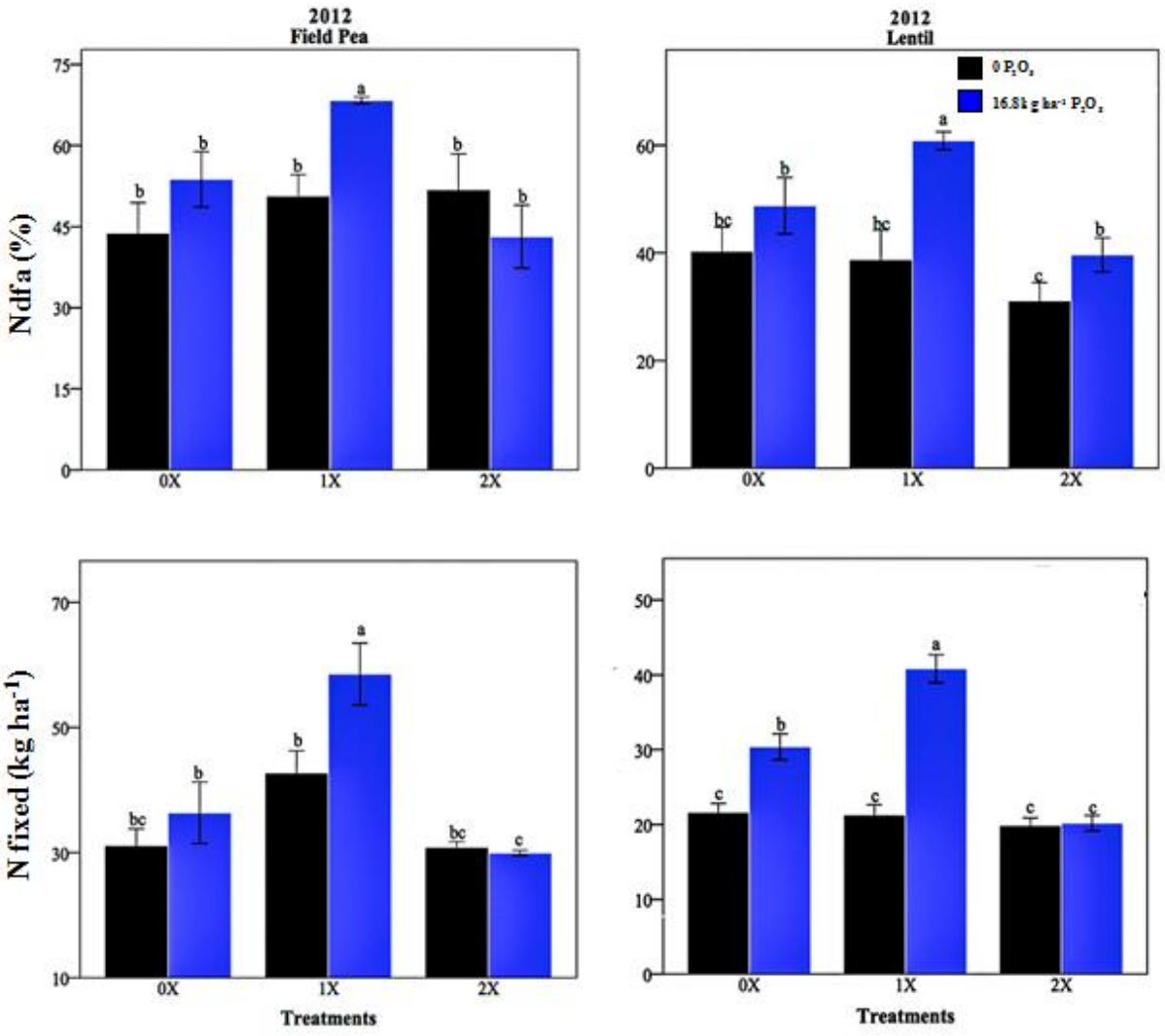


Fig 4.14. Mean percent N directly fixed from atmosphere (%Ndfa) and biologically fixed N of field pea and lentil in 2012 (averaged across all sites for field pea and at Stewart Valley for lentil). Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

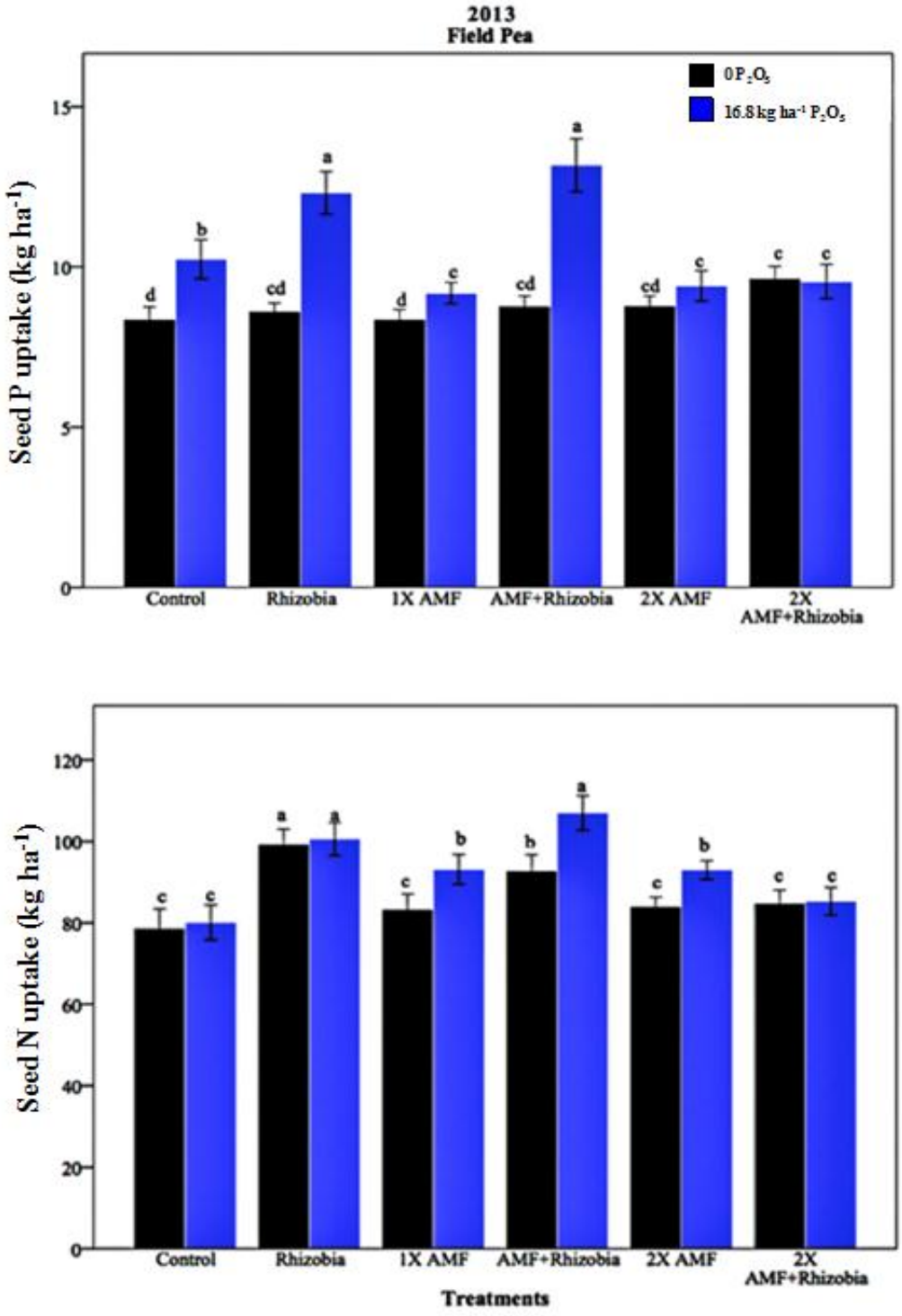


Fig 4.15. Mean percent N directly fixed from atmosphere (%Ndfa) and biologically fixed N of field pea in 2013 (averaged across all sites). Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

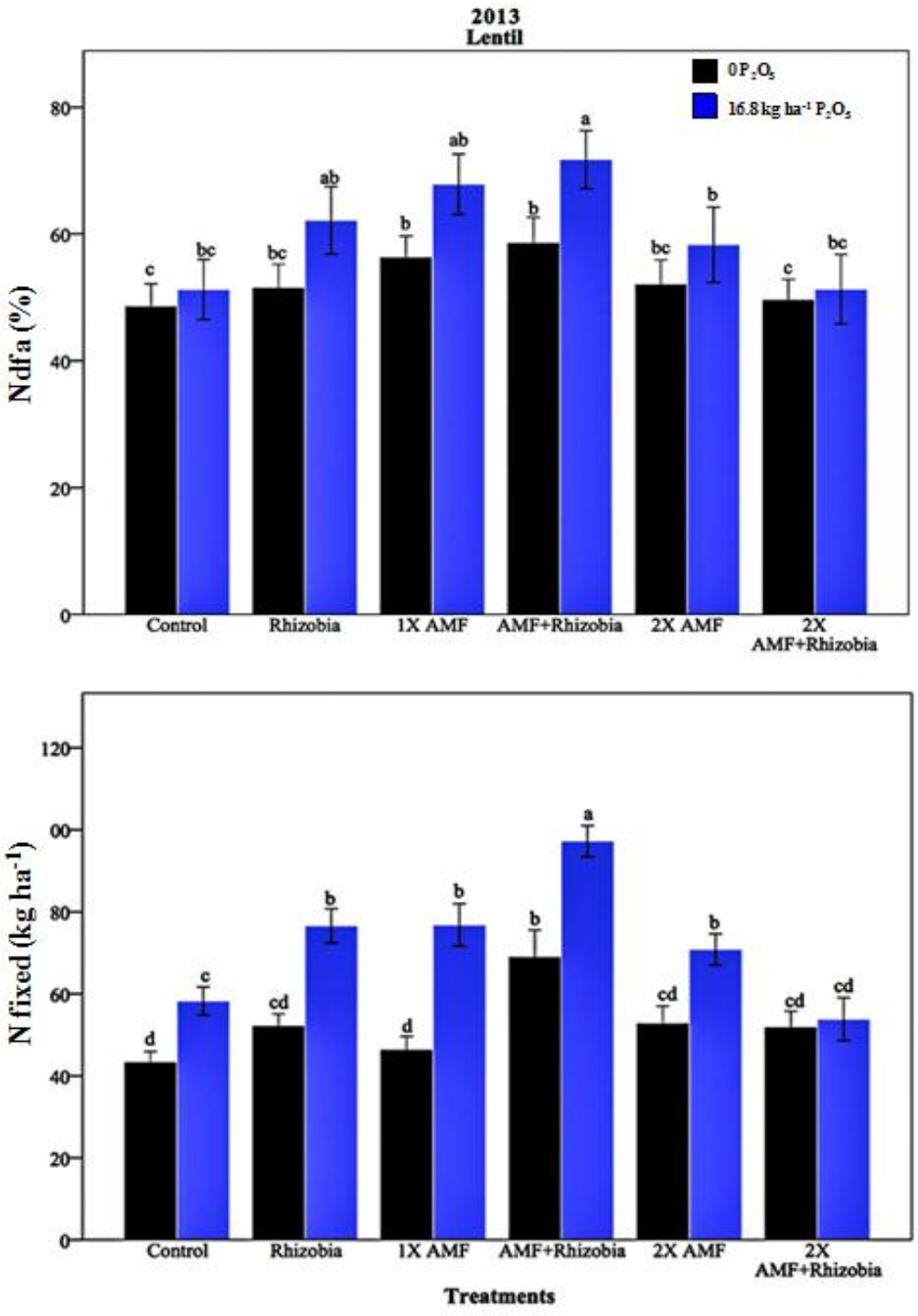


Fig 4.16. Mean percent N directly fixed from atmosphere (%Ndfa) and biologically fixed N of lentil at Stewart Valley in 2013. Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

The ANOVA of the means of % Ndfa and total N fixed are presented in Table 4.9. Class contrast effects for different rates of AMF application in both the host crops were observed to be variable. In 2012, significant differences were observed between 0X AMF and 1X AMF in pea for %Ndfa and total N fixed, but in lentil, differences were observed only for total N fixed. Significant AMF effects were observed for total N fixed in lentil, while P significantly affected total N fixed in both host crops and %Ndfa in field pea. Interaction effects of AMF and P were significant for %Ndfa and total fixed N in field pea and lentil. In 2013, for field pea, application of AMF significantly affected %Ndfa and total N fixed whereas *Rhizobium* was a significant source of variation for %Ndfa. Application of P fertilizer and interactions between AMF and P, AMF and *Rhizobium*, P and *Rhizobium* and, AMF, P and *Rhizobium* significantly affected %Ndfa and total biologically fixed N. Significant class contrast effects were observed for %Ndfa and total N fixed in pea between 0X AMF and 1X AMF. In lentil, application of P, interaction between AMF and P, and AMF, P and *Rhizobium* significantly affected %Ndfa and total biologically fixed N. Class contrast effects for different rates of application of AMF were observed only between 0X AMF and 1X AMF for total fixed N.

Table 4.9. Combined site analyses of the impact of AMF, P treatments in 2012, and AMF *Rhizobium* and phosphorus treatments in 2013 on %Ndfa and total biologically fixed N of field pea and lentil.

Treatments[†]	Pea				Lentil			
	% Ndfa		Total N fixed		% Ndfa		Total N fixed	
	F[†]	*p*[†]	F	*p*	F	*p*	F	*p*
2012 Field Season								
AMF[‡]	0.7	0.263	2.3	0.062	0.9	0.308	3.7	**0.037**
P[§]	0.4	0.364	6.1	**0.039**	5.1	**0.028**	4.2	**0.031**
AMF × P[¶]	8.1	**0.045**	7.6	**0.043**	4.7	**0.031**	5.8	**0.026**
Contrast[#]								
0X AMF vs 1X, 2X AMF	0.7	0.445	2.5	0.079	0.4	0.821	3.9	**0.042**
0X AMF vs 1X AMF	4.6	**0.046**	15.9	**0.004**	1.9	0.177	7.1	**0.019**
0X AMF vs 2X AMF	0.1	0.672	0.3	0.861	0.8	0.194	2.0	0.078
2013 Field Season								
AMF	3.9	**0.043**	7.5	**0.029**	1.9	0.194	1.8	0.210
P	7.3	**0.044**	4.2	**0.033**	4.7	**0.042**	5.7	**0.031**
Rhizobium[††]	3.6	**0.045**	2.0	0.074	1.5	0.201	1.5	0.113
AMF × P	6.3	**0.029**	5.3	**0.039**	4.0	**0.049**	6.0	**0.021**
AMF × *Rhizobium*[‡‡]	6.3	**0.046**	6.5	**0.026**	1.7	0.127	2.1	0.181
P × *Rhizobium*[§§]	3.6	**0.045**	6.0	**0.039**	0.5	0.472	1.5	0.601
AMF × P × *Rhizobium*[¶¶]	7.1	**0.036**	9.2	**0.029**	3.8	**0.042**	4.1	**0.031**
Contrast								
0X AMF vs 1X, 2X AMF	1.8	0.371	5.3	**0.037**	0.5	0.661	0.9	0.301
0X AMF vs 1X AMF	7.0	**0.035**	13.1	**0.009**	0.9	0.292	4.1	**0.044**
0X AMF vs 2X AMF	1.1	0.371	0.9	0.883	0.1	0.482	0.1	0.661

† F and *p* values for treatment effects and interaction terms and orthogonal comparison derived from an ANOVA ($p<0.05$).

‡ Three levels of AMF treatment are 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.

§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.

¶ AMF and P interactions.

Orthogonal class contrast = Class comparison of AMF application rates (7.5 kg ha^{-1} and 15 kg ha^{-1}, or average of 7.5 kg ha^{-1} and 15 kg ha^{-1}) as a class vs. none applied.

†† In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, control and recommended rate were applied.

‡‡ AMF and *Rhizobium* interactions.

§§ P and *Rhizobium* interactions.

¶¶ AMF, P and *Rhizobium* interactions.

4.6. Effects of Inoculation on AMF Communities of Field pea and Lentil Roots

A total of 155 OTUs were generated, identified and plotted on a phylogenetic (Appendix B). Phylogenetic analysis of the OTUs for the entire experiment showed that most of the *Glomeromycota* OTUs were related to 6 different genera. The most abundant OTUs were from the genera *Claroideoglomus* (56/155) while 31 other OTUs were closely related to *Rhizophagus*. In total, 9 OTUs were affiliated with genera *Funneliformis*, 10 members of genera *Diversispora*, 20 members of *Septoglomus* and finally 27 members of genera *Glomus* were identified. In 2012, at Kelvington, *Rhizophagus* (commercial inoculant OTU) were absent in field pea roots of the control treatment plants, while they dominated the roots of plants inoculated with the recommended rate of AMF + P (almost 50% relative frequency). Interestingly their dominance seemed to decline when either AMF was added without P or AMF treatment rate was increased to 2X. In Stewart Valley, *Rhizophagus* was present in the roots of the control treatment field pea and lentil plants (30-40% frequency), and it seemed to decline when P was added. In both the host plant roots varied results were observed when AMF was applied at the recommended rate along with P as well as when the application rate was increased to 2X. Similarly in 2013, sampled roots from the control treatments in Stewart Valley demonstrated high levels of *Rhizophagus* (commercial inoculant OTU) and the relative frequency of it seemed to be inconsistent across the applied treatments in both the host crops. In Outlook, *Rhizophagus* OTUs were largely absent in the roots except when AMF was applied at 2X rate combined with rhizobia and P. The treatment results were largely inconsistent across the different sites and years and could not be correlated with the agronomic results. Results are presented in Appendix B.

5. DISCUSSION

5.1 Effects of Dual Inoculation on Nodulation and AMF Colonization

The treatments responded in a relatively consistent manner across the various sites of the same year, despite differences in soil characteristics, site and treatments interaction effects were co-directionally patterned. A significant positive response was observed on nodulation in both field pea and lentil when co-inoculated with mycorrhizal and rhizobial inoculants as compared to the un-inoculated or single inoculated controls for both field pea and lentil during both growing seasons (Section 4, Table 4.3, Figures 4.2 and 4.3). These responses were observed to be pronounced when the plants were dually inoculated together with P fertilizer application. These results are strongly supported by several workers who reported similar increased nodulation under dual inoculation conditions in other legume species such as cowpea (*Vigna ungulicata*), soybean (*Glycine max*), pigeon pea (*Cajanus Cajun*), chickpea (*Cicer arietinum*) and faba bean (*Vicia faba*) (Ames and Bethlenfalvay, 1987; Ianson and Linderman, 1993; Jia et al., 2004; Antunes et al., 2006c; Nautiyal et al., 2010). In contrast, Barea and Azcon-Aguilar (1983) reported that the increased nutrient status of the symbiotic host due to AMF colonization might inhibit rhizobial infection and nodule formation. Bethlenfalvay et al. (1985) reported antagonistic interactions between mycorrhizal and rhizobial partners if one of them is pre-established in soybean-*Glomus*-*Rhizobium* symbiosis. Catford et al. (2003, 2006) found similar systemically regulated antagonistic effects in alfalfa plants when pre-inoculated with *Sinorhizobium meliloti*. They suggested the suppressive effect of one pre-established symbiotic partner on the other could be attributed to competition for limited carbohydrates and nutrients in the roots. Interestingly, these researchers reported that such competitive interactions do not inhibit plant growth parameters. The results of the current study also are in contrast to a study conducted by Vasquez et al. (2000), which showed that nodulation of *Medicago sativa* decreased with increasing AMF infection.

In the present study, strong AMF and P, and AMF, P and *Rhizobium* interaction effect on nodulation was observed in both crops over both cropping seasons (Table 4.1). Significantly higher nodulation was observed in dually inoculated plants fertilised with P as compared to

plants inoculated solely with *Rhizobium*, together with P fertilizer. A significant P fertilizer effect on nodulation was detected in dually inoculated plants where P fertilizer was applied. It should be noted that all the experimental sites had baseline P levels of <50 mg kg^{-1}, which according to Schubert and Hayman (1986), would be conducive for AMF colonization. It has been established that the *Rhizobium*-legume symbiotic association is energy intensive and the nodules have high levels of functional ATP and thereby P requirements (Barea and Azcon-Aguilar, 1983; Almeida et al., 2000; Scheublin et al., 2004). Olivera et al. (2004) reported that an increase in the P supplied to host plants led to a four-fold increase in nodule mass. Schulze (2004) suggested that due to complex biochemical and physiological factors affecting the tripartite association, the development of mycorrhizal association in legumes could be advantageous for the plants due to the efficient supply of P under nutrient poor conditions to support nodulation. Previous studies by Bethlenfalvay et al. (1985) report that higher levels of P in nodulated roots resulted in greater nodule activity and sugar depletion, indicating higher microbial activity as well as a competitive P-sensitive sink. Thus, the increased nodule numbers observed during dual inoculation can be attributed to improved P nutrition resulting from AM colonization, initiating a chain of biochemical and physiological cross-reactions activity among the tripartite symbiotic partners, thereby increasing host performance.

A significant depression in nodulation was observed in both field pea and lentil under the highest rate (2X the recommended field rate) of AMF application compared to dual inoculation at the recommended field rate. It can be considered that under limited nutrient supply and higher number of infective propagules, the host could be failing to support the tripartite association, causing the symbiotic associations be a net "resource drain". Literature on photosynthate allocation for dual symbiotic association illustrates that AMF and *Rhizobium* compete for the same source of C from the host and the combined drain is substantial with estimates of photosynthate allocation ranging from 10%-23% for AMF and 6%-30% for *Rhizobium* (Snellgrove et al., 1982; Kucey and Paul, 1981, 1982; Harris et al., 1985; Provorov and Tikhonovich, 2003). It can be argued that the "resource drain" was not the case in the current study, since the depression in growth parameters was consistent in P fertilized conditions and a similar depression was not observed for AMF colonization (Section 4, Table 4.3, Figures 4.2 and 4.3). However, Brown and Bethlenfalvay (1988) reported that AMF usually show a competitive advantage for carbohydrate allocation over *Rhizobium* under dual inoculation in terms of

infection levels. Research indicates that C allocation of the respective symbionts might be dependent upon their developmental stage, the cross-dependence on each other or P-supply levels (Mortimer et al., 2008). Morever, there is a lack of literature regarding the strength of individual symbiont C, and the cumulative effect of the two symbionts on host C-economy under different levels of inoculum application.

Another plausible explanation for the impact of inoculation on nodulation and AMF colonization could be under the conditions of higher AMF propagules, molecular and biochemical level cross talking between the two symbiotic partners might be activating a negative feedback mechanism for nodule organogenesis, thereby effectively inhibiting nodulation. Popp et al. (2014) identified a common symbiotic gene *MYCREM* that has co-evolved with the ability of legumes to establish root nodule symbiosis but gets specifically induced in cells containing arbuscules. In their study, knock-off *mycrem* mutants failed to develop arbuscules but were prolific nodulators under co-inoculation conditions. Of particular interest, when *MYCREM* was continuously over-expressed in cells, it repressed root nodule symbiosis. Further studies in plants where the calcium- and calmodulin-dependent kinase (CCaMK) had undergone point mutation at sites snf1 and snf2 (thereby triggering nodulation in the absence of rhizobia), led them to conclude that *MYCREM* acts as a controlled suppressor for nodule organogenesis when the host is infected with AMF.

Percentage of root length colonized by AMF in both the crops was not affected by the treatments. Workers reported similar findings during co-inoculation with AMF and *Rhizobium* in legume species (Bagyaraj et al., 1979; Kucey and Paul, 1982; Antunnes et al., 2006a; Tajini et al., 2012). Contrasting results have also been reported, where enhanced AMF colonization under co-inoculation conditions was observed (Pacovsky et al., 1986; Xie et al., 1995; Oldryod et al., 2005; Tavasolee et al., 2011). Chaturvedi and Singh (1986) reported enhanced mycorrhization in roots of chickpea (*Cicer arietinum* L.) under combined inoculation with *Rhizobium* and AMF. Xavier and Germida (2002, 2003) observed enhanced AMF colonization when compatible *Rhizobium* strain and AMF species were co-inoculated in both field pea and lentil in growth chamber conditions. On the other hand, Pearson et al. (1993) reported suppression of mycorrhization in the presence of two microsymbionts presumably due to competition for carbohydrates.

Antunes et al. (2006c) conducted a dual inoculation experiment with Nod^+ and Nod^- soybean plants under field conditions, and concluded that AMF colonization in legumes was not affected by the presence of the bacterial symbiont though nodulation was significantly higher in mycorrhized roots. They effectively argued that the lack of significant differences in AMF infection among treatments under field conditions could be due to high infectivity of indigenous AMF well adapted to their field environment, in combination with the higher mycorrhiza dependence of legumes. In the current study, the sites of field trials had indigenous multi-species AMF population ranging from 74 to 117 spores 100 g^{-1} of soil, while the commercial inoculant carries about 142 viable spores g^{-1} of only *Rhizophagus irregularis* (www.mykepro.com). It was rather surprising that the application of a single species inoculum at such high rates did not cause a significant change in AMF colonization levels. The post harvest MPN assay conducted in growth chamber conditions yielded a significant increase in AMF infection for the AMF treatments (Table A.4, 4.5, A.6). It must be considered that in natural ecosystems different microorganisms co-evolved over several millennia and in agricultural systems, individual AMF may be preferentially selected by crops due to crop management strategies.

Several studies argue the strong influence of P levels and host P status on AMF infection levels (Withers et al., 2001; De Clerck et al., 2003; Kogelmann et al., 2004). Schubert and Hayman (1986) declared AMF colonization was most favoured when soil P levels where less than 50 mg kg^{-1} and colonization is ineffective at levels of 100 mg kg^{-1} or higher. In the current study, the indigenous and introduced inoculum was indifferent to the baseline as well as fertilized P levels. The base line soil test P levels prior to fertilizaton varied between 3.3. to 38.5 kg ha^{-1} (i.e. 1.6 to 12.83 mg kg^{-1}) of P while the fertilized levels i.e. baseline levels soil test P + constant level of P fertilization ranged from 20.1 to 55.3 kg ha^{-1} (10.05 to 18.43 mg kg^{-1}) of P. Researchers reported varied AMF infection results under different P levels in field conditions. Jensen and Jakobsen (1980) reported highest AM colonisation at the sites with lowest soil P and reduced AM colonization due to application of P fertilizers. Workers reported low AMF colonization in soils with low available P levels in response to both native AMF and introduced inoculum (Ryan et al., 2002; Sainz et al., 1998). At the same time, high AMF infection levels were observed in soils with high available P by several researchers (Khalil et al., 1992; Vosatka, 1995; Gavito and Varela, 1995). Hamel et al. (1994) demonstrated under field conditions that AM fungal population and colonization levels were not affected by the application of P fertilizers

applied at different rates in barley (*Hordeum vulgare*). Similar results were also reported in ryegrass by Jasper et al. (1979). In the current study, soil P levels (baseline and fertilized) did not result in any significant effects AMF colonization under single or when dually inoculated with rhizobial fertilizer in both field pea and lentil.

It should also be noted that the absence of AMF colonization differences in co-inoculation couldn't be interpreted as lack of interaction among the microsymbionts. Nodule organogenesis causes an alteration in plant physiological, biochemical and systematic properties. Scheublin et al. (2004) suggested that due to the different nutrient and biochemical demands of nodules compared to the roots, AM species colonizing may be different in roots and nodules. Hence, the presence of *Rhizobium* in the plant roots could be modifying the AMF community colonizing the roots if not the colonization percent itself. Reciprocal interactive effects of AMF and *Rhizobium* in terms of colonization and nodulation was not detected; however, reciprocal interactions at molecular and biochemical levels cannot be ruled out. The intimate interactions between the microsymbionts and their individual relationship with host can be manifested in other areas of host parameters, apart from infection levels.

5.2. Effects of Inoculation on Crop Biomass and Nutrient Uptake

Arbuscular mycorrhiza fungi play a significant role in crop P uptake and P use efficiency, and are a main controlling factor in the tripartite symbiotic association, thereby influencing crop growth and nutrient uptake (Graham, 2000; Koide et al., 2000). Varied treatment responses in terms of crop growth parameters were observed across the different sites in 2012 and 2013. In 2012, application of mycorrhizae at the recommended rate showed a general increase in biomass, but doubling the application rate caused suppression in both host crops. Significant biomass increases were observed in response to AMF, P and *Rhizobium* interactions in 2013 for both pea and lentil (Tables 4.4.). A similar significant enhancement in N and P uptake by plants was observed when dually inoculated with P in both pea and lentil (Tables 4.5 and 4.6).

The findings of this field study are consistent with observations in previous studies that emphasize the effect of tripartite association on growth and nutrition of legumes (Vejsadova et al., 1993; Ianson and Linderman, 1993; Ruiz-Lozano and Azcon, 1993; Saxena et al., 1997; Nwoko and Sanginga, 1999; Xavier and Germida, 2002, 2003; Geneva et al., 2006). Ahmad (1995) declared that symbiotic efficiency of the AMF species and *Rhizobium* strain in co-

inoculation condition was dependent on the particular combination of co-symbionts and the combination thereby affects growth, yield and nutrition of the host plant.

Xavier and Germida (2002, 2003) demonstrated the efficacy of both "effective" and "ineffective" combinations of *Rhizobium* strains with AMF species in enhancing growth and nutrient uptake for both pea and lentil in growth chamber studies. They observed enhanced growth, yield and nutrient content in plants when co-inoculated with compatible AMF species and *Rhizobium* strains.

The findings demonstrate that plants benefited from the inoculation of AMF or/and rhizobia, while P addition mediated the growth and nutrient responses with each symbiont combination. Specific P response was evident when significant differences in growth and N and P uptake were observed in plants with the same combination of AMF and rhizobia between fertilizer addition treatments. The observed P response was irrespective of AMF colonization levels and agrees with Xavier and Germida (2003) that P uptake in mycorrhized roots may not be directly related to AMF colonization and could be mediated by the external mycelium. The influence of different forms of P (organic or inorganic) in soil, its availability to plant roots and uptake by AMF colonized plants is still under active research (Bu¨nemann et al., 2011).

Mycorrhizal symbiosis influences the P nutrition of the host plant by increasing the surface area for soil exploration and making inaccessible nutrients available to the plants (Moawad and Vlek, 1997; Smith and Read, 2008), improving uptake of P through more effective AM pathway, and by reducing the impact of depleting P ion transporters around the root zone (Smith and Smith, 2011). Marcel et al. (2008) noted that tripartite symbiotic partners can synergistically interact in nutrient poor ecosystems for the acquisition of macronutrients. There is a general consensus that AMF enhances P uptake and that is often associated with better N fixation and growth (Ruiz-Lozano and Azcon, 1993; Vosatka, 1995; Ibibijen et al., 1996; Koide et al., 2000; Xavier and Germida, 2002, 2003). Gavito et al. (2000) proposed that the improved nutrition of the mycorrhizal legumes led to enhanced N-fixation, thereby resulting in the dual symbiotic plants having greater growth. Extraradical hyphae of AMF are also capable of scavenging for N and can directly contribute in N uptake (Vazquez et al., 2001). In their co-inoculation study, they reported effective N acquisition in dually inoculated roots even under reduced nodulation. They declared that under a high N supply, biological N fixing capacity by

Sinorhizobium was reduced in AM plants, whereas the AM fungal extraradical mycelium may continue to contribute efficiently to the N uptake from the soil even at high N levels. This idea of interactions between the partners has been elaborated on by several workers (Azcón et al., 1992; Johansen et al., 1993; Cuenca and Azcón, 1994; Johansen et al., 1994; Azcón et al., 1996; Mäder et al., 2000; Govindarajulu et al., 2005). Their findings reported that nitrate reductase activity responsible for nitrate uptake and assimilation was enhanced under AM colonization irrespective of N supply or nodulation and that AMF play an important role in the N nutrition of plants whether in single or dual inoculation condition.

The results demonstrated enhanced growth, and N and P uptake associated with dual inoculation and agree with previous research that the effect of mycorrhizae and rhizobial inoculation on plants (both independent and dual) is dependent upon the biotic (co-symbionts) and abiotic (fertilizer) conditions of the environment (Marulanda et al., 2006; Mortimer et al., 2008). The increased biomass N and P in dually inoculated plants compared to control or singly inoculated (Figures 4.8., 4.9. and 4.10.) can be attributed to the role of AMF hyphae enhancing access and uptake of P from otherwise unavailable sources. The magnitude of the increases in biomass production was dependent on the P application level. The findings are consistent with other studies that find plant responses to the effects of nutrient addition on plant–AMF (Vogelsang et al., 2006) and plant–rhizobia interactions (Heath et al., 2010) are dependent upon host–symbiont combination. It was also observed that the higher magnitude of treatment responses in lentil compared to pea is consistent with phytochamber experiment results of Xavier and Germida (2003). Research regarding the effects of AM symbiosis on plant growth under different levels of P application has been conducted under controlled and greenhouse conditions, and there has been little research regarding the effects of AM symbiosis on plant growth under field conditions, with different levels of inoculum and P application (Daei et al., 2009; Mardukhi et al., 2011).

Growth and nutrient uptake depression was observed in treatments with high AMF inoculum application, even with P application. These plants also were associated with high AM colonization, but colonization did not consistently confer benefits when the AMF application rate was twice the recommended rate. It can be explained by reduced P delivery via the direct pathway through roots due to AM colonization, but it was not compensated by uptake via the AM pathway, leading to reduced total plant P uptake (Smith et al., 2009; Smith and Smith,

2011). The cumulative effect of the high AMF inoculation rate led to lower nodulation, N uptake and depression in overall growth. The high fungal C cost associated with maintaining the AM symbiosis coupled with lack of P "benefit" could be the primary cause of growth depression (Grace et al., 2009; Smith et al., 2009).

5.3. Effects of Inoculation on Seed Yield and Nutrient Concentration

Research has shown varied results regarding yield impact and seed nutrient content when legumes are inoculated with AMF with or without *Rhizobium*. Early workers reported that the inoculation of legumes with AMF (Islam and Ayanaba, 1981; Ganry et al., 1982) as well as dual inoculation with AMF and *Rhizobium* can improve seed yield (Badr El-Din and Moawad, 1988). In contrast, some workers reported neutral effects of dual inoculation on seed yield while compared to single inoculation (Bagyaraj et al., 1979).

In the study, no significant yield differences were observed in 2012 and 2013 for both pea and lentil crops with the application of AMF + P treatment or dual application of AMF and rhizobia combined with P (Table 4.7). Seed N and P uptake of legumes were significantly enhanced compared to the uninoculated control under field conditions, with differences between treatments most pronounced when P fertilizer was applied (Table 4.8). Other workers have reported enhanced yield under dual inoculation conditions. For example, Pacovsky et al. (1986) reported a significant improvement in seed yield in soybean when compatible *Bradyrhizobium* strains were paired with AMF. Thiagarajan et al. (1992) declared similar results while working with cowpea. Mehdi et al. (2006) inoculated lentil with different AMF species (*G. mosseae and G. intraradices*), rhizobia strains (*R. leguminosarum bv. viciae*), and P (superphosphate and phosphate rock) fertilizers, and observed considerably enhanced yield, P and N contents of seeds under dual inoculation and P addition. There is considerable literature reporting enhanced seed quality parameter, when host crops are inoculated with arbuscular mycorrhizal fungi with or without rhizobia. Workers report increases in content and composition of many substances in the seeds or fruits of their host plants, e.g., essential oils (Kapoor et al., 2002, 2004), proteins and lipids (Bethlenfalvay et al., 1994), bioactive compounds (Venkateswarlu et al., 2008), or economically important secondary metabolites (Yuan et al., 2007).

Xavier and Germida (2002, 2003) observed enhanced seed yield and seed nutrient content when they inoculated pea and lentil host plants with different combinations of AMF and

Rhizobium strains under growth chamber conditions. They suggested that the yield increment under certain successful combinations was due to enhanced N and P uptake attributed to the tripartite symbiotic association, which led to healthier plants. They also observed higher magnitude of responses in lentil as compared to pea for similar combinations, which is consistent with the field experiment. This phenomenon can be explained by higher mycorrhizal dependency of lentil to fulfill their P and other nutrient requirements compared to pea. Enhanced overall N and P uptake was observed in both the host crops under dual inoculation (Section 4.3, Tables 4.5 and 4.6, Figures 4.4, 4.5., 4.6., 4.7. and 4.8.), but it did not translate into higher seed yields. Enhanced seed nutrient content was observed in response to the treatments (Section 4.4.2, Table 4.8, Figures 4.11., 4.12 and 4.13.). These results are consistent with observations noted by many workers, under different host and growing conditions (Ames et al., 1991; Azcon et al., 1991; Vejsadova et al., 1992; Ahmad 1995; Redecker et al., 1997). On the other hand neutral and sometimes antagonistic yield responses were observed by Rydlova and colleagues (2011) between AMF and *Sinorhizobium* on flax (a non-legume plant) yield on spoil bank clay, suggesting that the interactions were species specific. Researchers maintain that any potential neutral/antagonistic interaction in tripartite association could be context dependent and more likely suggest low mycorrhizal dependency, biotic/abiotic limitation or incompatible pairing of symbiotic partners (Francis and Read, 1995; Toro et al., 1997; Valdenegro et al., 2001; Klironomos, 2003; Jones and Smith, 2004).

Kaschuck et al. (2010) conducted a meta-analysis of 348 data points from published studies with 12 legume species to test whether yield, seed protein and lipid mass fractions are affected by tripartite symbiosis. Significant yield increases, of up to 45%, were observed due to AM fungal and/or rhizobial inoculation under pot experimental conditions. They concluded that the AM fungal and rhizobial inoculum, either applied in single or in dual inoculation, did not cause significant yield response in legumes in field experiments. It was implied that it could be due to the difficulty in ensuring an AMF-free control in the field rather than an actual lack of response to introduced inocula.

5.4. Effects of Inoculation on Biologically Fixed Nitrogen

Nitrogen-fixing bacteria are known to improve the bioavailability of N to plants, and this capability may be enhanced in presence of mycorrhized roots (Barea et al., 2002c). Previous

research has established that the mycorrhizal and root nodule symbioses are typically synergistic both with regard to infection rate and their impact on mineral nutrition and growth of the plant.

The results from the field trials over 2012 and 2013 yielded significant results for both the host crops in terms of percent N directly fixed from atmosphere (%Ndfa) and total N fixed. Significantly higher percent Ndfa and total N fixed was noted in pea and lentil plants when inoculated with 1X AMF + P in 2012, compared to control treatments (Table 4.7, Figure 4.14). In 2013, interestingly significant enhancement in biological N fixation in all the treatments was observed compared to control (Table 4.7, Figures 4.15 and 4.16). Increasing the application rate of AMF inoculant caused a decline in biological N fixation in both pea and lentil. The results are consistent with research from several workers, who reported a general increase or a significant enhancement in biological N fixation in dually inoculated roots of plants (Kucey and Paul, 1982; Kawai and Yamamoto, 1986; Ames and Bethlenfalvay, 1987; Louis and Lim, 1988; Schoeneberger et al., 1989; Ianson and Linderman., 1993; Fitter and Garbaye, 1994; Smith and Read, 1997; Karpenstein-Machan and Stuelpnagel, 2000; Goss et al., 2002; Barea et al., 2002c; Jia et al., 2004; Karandashov and Bucher, 2005; Antunes et al., 2006b; Nautiyal et al., 2010). Schoeneberger et al. (1989) observed larger responses in legume fixed N when the hosts were dually inoculated, compared with the response of the individual components. In their study, they observed a 4.4-fold increase in biologically fixed N in dually inoculated clover plants. They attributed the enhanced response to AM dependencies of legumes for P acquisition as well as root architecture. Toro and colleagues (1998) used the $^{15}N/^{14}N$ ratio in plant shoots to show that N_2 fixation rates in *Rhizobium meliloti* inoculated mycorrhizal alfalfa plants were higher than the corresponding rates in non-mycorrhizal plants. Requena et al. (2001) conducted an experiment with dual inoculation of AMF and *Rhizobium* in degraded field conditions with *Anthyllis cytisoides* L., a drought-tolerant legume, as the host. They recorded enhanced P acquisition and biological N fixation by the host legume. The benefits also included increased available P, N and organic matter, and better soil structure to support the microbial community. They also observed N-transfer to non-N-fixing plants through hyphal network and an accelerated natural succession in vegetation grown in association with the inoculated legume. Goss and de Varennes (2002), reported similar results while working with disturbed and undisturbed soil. In their study they co-inoculated *Bradyrhizobium* and indigenous AMF in soybean roots, and observed higher AMF colonization, with hyphal, arbuscular and vesicular frequencies as well as higher nodulation and

biological N fixation in undisturbed soil. They concluded that disturbing the soil impaired the rate and extent of colonization, which in turn impacted the tripartite association and N-fixation. Enhanced N-fixation in co-inoculation conditions also been reported by Xavier and Germida (2003) with pea and lentil in phytochamber experiments.

Arbuscular mycorrhizal fungi contribute to enhanced nutrient uptake by decomposing organic compounds, P acquisition and other micronutrients, thereby increasing the nutrient status of the host plants. Improved P uptake of dually inoculated plants, later translates as more energy available for N fixation by rhizobia in nodules. Enhanced nodulation and biological N fixation lead to higher levels of P and N in the plant biomass, thus healthier plants. The overall general plant growth improvement could be indirectly affecting the N_2-fixing system of hosts. Better plant health parameters (higher nodulation, P and N uptake and biologically fixed N) result in a better crops stand in the field, there by an overall increase in yield. In support of this hypothesis, workers reported comparable enhanced N-fixing ability in mycorrhizal plants supplied with a readily available P source (Carling et al., 1978, Asimi et al., 1980 and George et al., 1995)

Antunes et al. (2006a and 2006b) reported that the presence of both *G. clarum* and *Bradyrhizobium japonicum* in soybean plants (*Glycine max* (L.) Merr.) caused an accumulation of flavonoids in soybean roots. Flavonoids are known to be the primary signal compounds associated with the establishment of the tripartite symbiosis in legumes. They further observed that changes in the signalling patterns, particularly daidzein, genistein and coumestrol led to an enhanced nodulation in soybean plants colonized by both AMF and *Bradyrhizobium* increased N_2 fixation at flowering, compared to plants grown in soil inoculated only with *B. japonicum.*

Higher %Ndfa in field pea was noted when inoculated with only AMF in 2013; interestingly those plants also had higher nodulation. This could be explained by the presence of prolific native *Rhizobium* community in the soil. Consistent with the trends in the observations, a depression in biological N-fixation was observed at higher rates of AMF application (Figures 4.14., 4.15. and 4.16).

6. SUMMARY AND CONCLUSIONS

In the current study, a considerable diversity in responses to tripartite symbiotic association in legumes was noted between years and different years had different sites and field conditions. It is likely, therefore, that agronomically relevant impacts of the microbial associations are determined by several biotic (other microbial partners, pathogens) and abiotic (soil pH, nutrient availability, tillage, irrigation as well as environmental conditions. The physiological aspect of plant-microbiont symbioses in terms of benefits derived (macro and micro-nutrient supply) and costs of the association (supply of photosynthates) also needs to be considered. Assessing every individual parameter while controlling for the various factors under actual environmental conditions can be very difficult. Plants in the field will be colonized by several indigenous AMF, which co-evolved with them and could possibly be deriving some specific benefits contributing to the success of the inoculum.

Dual inoculation with commercial AMF fertilizer and *Rhizobium* in legume cropping systems can potentially benefit plant growth, grain yield and biological N fixation. Most of the studies regarding effects of microbial inoculum application on crop growth mainly focused on growth chamber conditions. Growth chamber experiements typically use relatively small rooting volumes and thus it is challenging to translate results to actual field performance. The current research described here examined the response of field pea and lentil grown in different field sites in Saskatchewan to the application of commercial AMF at two rates, alone and in combination with *Rhizobium* inoculants, when applied with and without P fertilizer. Commercial AMF inoculation is a recent development in Saskatchewan farming practices, and little is known about its effects on prairie soils. A review of previously published literature (Chapter 2) provided valuable ground work and justification for the actual field study (Chapter 3, 4 and 5).

The results of the field studies (Chapter 4) with two host crops and five different sites in Saskatchewan showed that dual inoculation of AMF and *Rhizobium* application enhances crop growth, nutrient uptake and biological N fixation, and the effect is significantly higher under P fertilizer application. The introduction of AMF inoculum along with *Rhizobium* in combination with P fertilizer rates at all the field sites in Saskatchewan showed effects on nodulating capacity, shoot nutrient uptake, seed nutrient quality and biological N fixation. Although some growth parameters were enhanced, these effects did not translate into enhanced seed yield. Specifically,

dual inoculation together with P fertilization did not cause significant differences in seed yields in either of the hosts.

Dual inoculation with addition of P tended to increase mid-season nutrient uptake in the host crops in both the cropping seasons. The effect on biomass yield was variable but it tended to be beneficial in both hosts. A significant AMF effect was also observed in terms of P uptake by the legumes, which was enhanced in co-inoculation treatments. A significant effect on nodulation and biological N fixation was observed in co-inoculation that suggests synergism among the commercial strains of AMF and *Rhizobium* inoculants. The interaction effects between AMF and *Rhizobium* were more dramatic in lentil as compared to field pea. This suggests host crop interactions and differences in microbial dependency among host species.

The effects of doubling the rate of AMF application were also evaluated (Chapter 4). Increasing the rate of AMF application, with and without *Rhizobium* yielded negative results, even with application of P fertilizer. A significant depression in crop growth and nutrient uptake was observed at higher rates of AMF application. The antagonistic effect was observed at both single and dual inoculation, with and without P application. It also caused reduced nodulation as well as depression in biologically fixed N. This effect was consistent at all the sites and in both host crops.

6.1 Implications and Recommendations

Mycorrhizal fungi are known to enhance crop plant growth, grain nutrient content and overall health. Mycorrhizal fungi are also known to enhance soil biochemical processes and affect microbial community composition. A commercial AMF fertilizer was used in field trials to assess the agronomic implications of co-inoculation with *Rhizobium* and P application in prairie soils. Application of mycorrhizal inoculum appeared to be effective in promoting some legume growth parameters and nutrient uptake in prairie soils, although responses tended to vary between sites and years. Importantly, despite observed increases in some growth parameters, no seed yield increases were observed for either field pea or lentil. These results suggest that although AMF inoculation holds promise for promoting plant growth characteristics in field pea and lentil, further research is required to determine any actual field agronomic benefits.

Previous studies for assessing the impact of AMF application has been conducted in growth chamber conditions and experiments that assessed the impacts of rates of inoculum were conducted in pot studies with controlled conditions. The rates of inoculum were also in terms of number of spores, which may or may not be infective. This study tested the efficacy of the commercially available AMF inoculum at double the rate of recommended application, under actual field conditions. Assessments from the trials suggest that increasing the application rates of AMF did not beneficially impact on the crop growth or productivity either in single or dual inoculation condition, irrespective of P application.

6.2 Future Research

Arbuscular mycorrhizal fungi are widely considered a very important biological resource in the soil and their contribution to biological, chemical and physical quality of soil has been widely researched and documented. They are also known to actively interact and modify their biotic and abiotic environment. Sanginga et al. (1999) claimed that mycorrhizal fungi could be the most important untapped poorly understood resource for nutrient acquisition and plant growth in agriculture. The basic mechanisms by which they interact with other symbiotic partners in their host and modify its physiology is still poorly understood, particularly in cases of agroecosytems where there are several factors playing a role in the biogeochemical cycles. Saskatchewan has several soil ecozones where the biotic and abiotic conditions vary greatly and these soils could be under different kinds of agricultural management practices (conventional, organic, tillage, no tillage etc), which can interact with biofertilizers such as the inoculants used in this study. Conventional agriculture practices, characterized by high levels of synthetic fertilizers, rotation with non-mycorrhizal plants (like canola) and the use of pesticides and fungicides, may negatively impact the indigenous microbial community structure and may modify the effect of the introduced inoculum.

Evaluation of the agronomic benefits under a wider variety of legumes, soil and environmental conditions, the interactions with other prevalent microbial inoculants and biofertilizers, and application rates should be investigated before widespread use of AMF as a biofertilizer in agricultural systems can be recommended in Saskatchewan. Nonetheless, the results from the field trials suggest that for legume crop combinations, inoculation of AMF and *Rhizobium* in combination with low P application can be beneficial.

In the field conditions, occasional depressions in biomass and crop yield were observed in both pea and lentil. The benefits of dual inoculation were highly dependent upon other biotic and abiotic factors. The 2012 and 2013 cropping seasons were uncharacteristically colder and wetter than the 30-year average, and the effect of climatic conditions, particularly during the crop establishment phase, cannot be ruled out. Understanding the effects of soil types, seasonal conditions and crop management practices on the microbial fertilizer is necessary to assess the utility of AMF and rhizobia as microbial inoculants in Saskatchewan. Microbial populations can vary according to seasonal changes and that variability also needs to be taken into account during application of microbial fertilizers.

Finally, it is imperative to understand the impact of long-term application of AMF and rhizobia simultaneously in legume crop systems on grain yield and soil quality of prairie soils. Research should also assess under what biotic and abiotic conditions inoculation with mycorrhizal fungi in agro-ecosystems can prove beneficial with the goal of discovering an optimum combination for co-inoculation with rhizobia in legumes. Attention should also be given to the idea that mycorrhizal inoculum quantity or quality could prove non-beneficial/antagonistic under certain conditions, thereby limiting productivity in agro-ecosystems. Although AMF fertilizers garnered attention with their success in horticulture and remediation systems, there is inadequate knowledge on the effects of their repeated applications in intensive systems as in agro-ecosystems.

7. REFERENCES

Adeleke, A. 2010. Effect of arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria on glomalin production. M.Sc, University of Saskatchewan, Saskatoon, SK.

Agrios, N.G. 2005. Plant Pathology, Fifth Edition, Elsevier, Amsterdam. p. 635

Ahmad, M.H. 1995. Compatibility and co-selection of vesicular arbuscular mycorrhizal fungi and rhizobia for tropical legumes, Crit. Rev. Biotech. 15:229–239.

Akiyama, K., and H. Hayashi. 2006. Strigolactones: chemical signals for fungal symbiosis and parasitic weeds in plant roots. Ann Bot. 97:925–931

Akiyama, K., K. Matsuzaki, and H. Hayashi. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature. 435:824–827

Al-Karaki, G.N. 2002. Field response of garlic inoculated with arbuscular mycorrhizal fungi to phosphorus fertilization. Journal of Plant Nutrition 25: 747-756.

Al-Karaki, G.N., and R.B. Clark. 1999. Varied rates of mycorrhizal inoculum on growth and nutrient acquisition by barley grown with drought stress. J. Plant Nutr. 22:1775-1784.

Al-Karaki, G.N., B. McMichael, and J. Zak. 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza14:263–269

Allen, O. N., and E. K. Allen. 1981. The Leguminosae. A source book of characteristics, uses and nodulation, University of Wisconsin Press, Wisconsin.

Almeida, J.P.F., U.A. Hartwig, M. Frehner, J. Nosberger, and A. Luscher. 2000. Evidence that P deficiency induces N feedback regulation of symbiotic N2 fixation in white clover (*Trifolium repens* L.). J. Exp. Bot. 51:1289–1297.

Ames, R. N., and G. J. Bethlenfalvay. 1987. Mycorrhizal fungi and the integration of plant and soil nutrient dynamics. J. Plant Nutr. 10:1313-1321.

Ames, R.N., T.R. Thiagarajan, M.H. Ahmad, W.A. McLaughlin. 1991. Co-selection of compatible rhizobia and vesicular-arbuscular mycorrhizal fungi for cowpea in sterilized and non-sterilized soils. Biology and Fertility of Soils 12:112-116.

Andrade, G., K.L. Mihara, R.G. Linderman, G.J. Bethlenfalvay. 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular mycorrhizal fungi. Plant Soil 192:71–79

Antunes, P.M., A. de Varennes, I. Rajcan, M.J. Goss. 2006a. Accumulation of specific flavonoids in soybean (*Glycine max* .L. Merr.) as a function of the early tripartite symbiosis with arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum*. Kirchner. Jordan. Soil Bio. & Biochem. 38:1234–1242.

Antunes, P.M., A. de Varennes, I. Rajcan, M.J. Goss. 2006b. Specific flavonoids as interconnecting signals in the tripartite symbiosis formed by arbuscular mycorrhizal fungi, *Bradyrhizobium japonicum* .Kirchner. Jordan and soybean (*Glycine max.* L. Merr.). Soil Bio. Biochem. 38:533–543.

Antunes, P.M., A. de Varennes, T. Zhang, M.J. Goss. 2006c. The tripartite symbiosis formed by indigenous arbuscular mycorrhizal fungi, *Bradyrhizobium japonicum* and soya bean under field conditions. J. Agron. Crop Sci. 192:373–378.

Artursson, V., R.D. Finlay, and J.K. Jansson. 2005. Combined bromodeox-yuridine immunocapture and terminal restriction fragment length polymorphism analysis highlights differences in the active soil bacterial metagenome due to *Glomus mosseae* inoculation or plant species. Environ. Microbiol. 7: 1952–1966

Artursson, V., R.D. Finlay, and J.K. Jansson. 2006. Interactions between Arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ. Microbiol. 8:1-10.

Asai, T. 1944. Die bedeutung der mikorrhiza für das pflanzenleben. Japanese J. Bot. 12:359-408.

Asimi, S., V. Gianinazzi-Pearson, and S. Gianinazzi. 1980. Influence of increasing soil-phosphorus levels on interactions between vesicular arbuscular mycorrhizae and *Rhizobium* in soybeans. Can. J. Bot. 58:2200-2205.

Auge´, R.M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza. 11: 3–42.

Auge´, R.M., 2004. Arbuscular mycorrhizae and soil/plant water relations. Can. J. Soil Sci. 84:373–381.

Ayres, K.W., D.F. Acton and J.G. Ellis. 1985. The Soils of the Swift Current Map Area 72J Saskatchewan, Saskatchewan Institute of Pedology Publication S6.

Azco´n-Aguilar, C., J.M. Barea. 1996. Arbuscular mycorrhizas and biocontrol of soil-borne plant pathogens, an overview of the biological mechanisms involved. Mycorrhiza 6:457–464.

Azcon, R, R. Rubio, J.M. Barea. 1991. Selective interactions between different species of mycorrhizal fungi and *Rhizobium meliloti* strains, and their effects on growth, N_2 fixation (N^{15}) in *Medicago sativa* at four salinity levels. New Phyto. 117:399-404.

Azcón, R, M. Gómez, R.M. Tobar. 1996. Physiological and nutritional responses by *Lactuca sativa* to nitrogen sources and mycorrhizal fungi under drought. Biol. Fert. Soils. 22:156–161.

Azcón, R., M. Gómez, and R. Tobar. 1992. Effects of nitrogen source on growth, nutrition, photosynthetic rate and nitrogen metabolism of mycorrhizal and phosphorus-fertilized plants of *Lactuca sativa* L. New Phytol. 121:227-234.

Azcón, R., R. Rubio, and J.M. Barea. 1991. Selective interactions between different species of mycorrhizal fungi and *Rhizobium meliloti* strains, and their effects on growth, N_2-fixation (^{15}N) and nutrition of *Medicago sativa* L. New Phytol. 117:399–404.

Azcon-Aguilar, C., and B. Bago. 1994. Physiological characteristics of the host plant promoting an undisturbed functioning of the mycorrhizal symbiosis. In: S. Gianinazzi and H. Schiiepp, Eds. Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems, ALS: Birkhiiuser, Basel, p. 47-60.

Azcón-Aguilar, C., and J.M. Barea. 1997. Applying mycorrhiza biotechnology to horticulture, significance and potentials. Scientia Horticulturae, 68:1-24.

Azcón-Aguilar, C., M.C. Jaizme-Vega, and C. Calvet. 2002. The contribution of arbuscular mycorrhizal fungi to the control of soil-borne plant pathogens. In S. Gianinazzi, H. Schüepp, J. M. Barea and K. Haselwandter .Eds. Mycorrhiza Technology in Agriculture, from Genes to Bioproducts. ALS Birkhäuser Verlag: Heidelberg, Germany. p. 187-197.

Badr, El-Din, S.M.S., and H. Moawad. 1988. Enhancement of nitrogen fixation in lentil, faba bean and soybean by dual inoculation with rhizobia and mycorrhizae. Plant Soil, 108:117-124.

Bagyaraj, D. J., 1992. Vesicular-arbuscular mycorrhiza: Application in agriculture. In: Norris, J. R. Read, D. J. and Varma. A.K. eds). Methods in Microbiology. Vol 24, Academic press, London. p. 259-373

Bagyaraj, D. J., A. Manjunath, and D. D. R. Reddy. 1979. Interaction of vesicular arbuscular mycorrhiza with root knot nematodes in tomato. Plant and Soil 51:397-403.

Bainard, L.D., Bainard, J.D., Hamel, C., and Gan, Y. 2014. Spatial and temporal structuring of arbuscular mycorrhizal communities is differentially influenced by abiotic factors and host crop in a semi-arid prairie agroecosystem. FEMS Microbiol. Ecol. 88: 333–344.

Banba, M., C. Gutjahr, A. Miyao, H. Hirochika, U. Paszkowski, H. Kouchi, and H. Imaizumi-Anraku. 2008. Divergence of evolutionary ways among common *sym* genes: CASTOR and CCaMK show functional conservation between two symbiosis systems and constitute the root of a common signaling pathway. Plant and Cell Physiol. 49:1659-1671.

Barea J.M., C. Azcon-Aguilar, and R. Azcon. 1993. Mycorrhiza and crops. Adv. Plant Pathol. 9: 167-189

Barea, J. M., and C. Azcón-Aguilar. 1983. Mycorrhizas and their significance in nodulating nitrogen fixing plants. Adv. Agron. 36:1-54.

Barea, J. M., M. Gryndler, Ph. Lemanceau, H. Schüepp, and R. Azcón. 2002b. The rhizosphere of mycorrhizal plants. In: Gianinazzi, S., H. Schüepp, J. M. Barea and K. Haselwandter, Eds. Mycorrhiza Technology in Agriculture, from Genes to Bioproducts. Basel, Switzerland, Birkhäuser Verlag. pp. 1-18.

Barea, J. M., M. Toro, M. O. Orozco, E. Campos, R. Azcón. 2002c. The application of isotopic P^{32} and N^{15} dilution techniques to evaluate the interactive effect of phosphate-solubilizing rhizobacteria, mycorrhizal fungi and *Rhizobium* to improve the agronomic efficiency of rock phosphate for legume crops. Nutr. Cycl. Agroecosys. 63:35–42.

Barea, J. M., R. Azcón, and C. Azcón-Aguilar. 2002a. Mycorrhizosphere interactions to improve plant fitness and soil quality. Antoine van Leeuwenhoek, 81, 343-351.

Barea, J., M. Pozo, R. Azcon, and C. Aguilar. 2005. Microbial co-operation in the rhizosphere. J. Exp. Bot 56:1761–1778

Barea, J.M. 1997. Mycorrhiza-bacteria interactions on plant growth promotion. In: Plant Growth Promoting Rhizobacteria. Ogoshi, A., K. Kobayashi, Y. Homma, F. Kodama, N. Kondo, and S. Akino, Eds. Paris, France: OECD Press. 150–158.

Barea, J.M., R. Azcon, and C. Azcon-Aguilar .1992. Vesicular–arbuscular mycorrhizal fungi in nitrogen-fixing systems. Methods Microbiol. 24:391–416

Barea, JM. 2000. Rhizosphere and mycorrhiza of field crops. In: Toutant J.P., E. Balazs, E. Galante, J.M. Lynch, J.S. Schepers, D. Werner, and P. A. Werry, Eds. Biological resource management: connecting science and policy, OECD. INRA Editions and Springer: Berlin Heidelberg New York. p. 110–125

Bethlenfalvay, G. J., and R.G. Linderman. 1992. Mycorrhizae in sustain- able agriculture. ASA Special publication no 54. Madison, Wis. p. VIII–XIII

Bethlenfalvay, G. J., K. L. Mihara, R. P. Schreiner. 1994. Mycorrhizae alter protein and lipid contents and yield of pea seeds. Crop Sci. 34:998–1003.

Bethlenfalvay, G. J., M. S. Brown, and A.E. Stafford. 1985. *Glycine-Glomus Rhizobium* symbiosis.II. Antagonistic effects between mycorrhizal colonization and nodulation. Plant Physiol. 79:1054- 1058.

Bethlenfalvay, G.I., and W.E. Newton. 1991. Agro-ecological aspects of the mycorrhizal, nitrogen-fixing legume symbiosis. In: The Rhizosphere and Plant Growth Keister, D. L. and P. B. Cregan, Eds. Kluwer Academic Publishers: Wageningen. p. 349-354.

Bharadwaj, D.P., P. Lundquist, and S. Alström. 2008. Arbuscular mycorrhizal fungal spore-associated bacteria affect mycorrhizal colonization, plant growth and potato pathogens. Soil Biol. and Biochem. 40:2494-2501.

Bianciotto, V., D. Minerdi, S. Perotto, and P. Bonfante. 1996. Cellular interactions between arbuscular mycorrhizal fungi and rhizosphere bacteria. Protoplasma. 193 (1-4):123-131.

Bianciotto, V., S. Andreotti, R. Balestrini, P. Bonfante, and S. Perotto. 2001. Extracellular polysaccharides are involved in the attachment of *Azospirillum brasilense* and *Rhizobium leguminosarum* to arbuscular mycorrhizal structures. Eur. J. Histochem., 45: 39-49.

Biró, B., K. Köves-Péchy, I. Vörös, T. Takács, P. Eggenberger, and R.J. Strasser. 2000. Interrelations between *Azospirillum* and *Rhizobium* nitrogen-fixers and arbuscular mycorrhizal fungi in the rhizosphere of alfalfa in sterile, AMF-free or normal soil conditions. Appl. Soil Ecol. 15:159-168.

Bolton, H., J.K. Frederickson, L.F. Elliott. 1992. Microbial ecology of the rhizosphere. In:Metting FB .ed. Soil Microbial Ecology, Marcel Dekker, New York. p 27–36.

Boucher, D. H. 1985. The idea of mutualism, past and future. In The Biology of Mutualism. Eds. D. H. Boucher. Croom Helm, London. p. 1–28.

Bowen, G.D. 1980. Misconceptions, concepts and approaches mycorhizosphere biology. In: Ellwood D.C., Hedger J.N, Latham MJ, Lyncb JM, Slater JM eds. Contemporary microbial ecology.London: Academic Press, 283-304

Bremer, E. and C. van Kessel. 1990. Appraisal of the nitrogen-15 natural abundance method for quantifying dinitrogen fixation. Soil Sci. Soc.Am.J. 54:404-411.

Bressan, W., J.O. Siqueira, C.A. Vasconcellos, A.A.C. Purcino. 2001. Fungos micorr´ızicos e fo´sforo, no crescimento, nos teores de nutrientes e na produc¸a˜o do sorgo e soja consorciados. Pesqui. Agropecu. Bras. 36: 315–323.

Brockwell, J., P.J. Bottomley, J.E. Thies. 1995. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. Plant Soil 174:143–180

Brown, M.S., S. Thamsurakul, and G.J. Bethlenfalvay. 1988. The Glycine-Glomus-Bradyrhizobium symbiosis. VIII. Phosphorus-use efficiency of CO_2 and N_2 fixation in mycorrhizal soybean. Physiol. Plant. 74:159-163.

Bu¨nemann, E.K., A. Obserson, E. Frossard, Eds. 2011. Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling. Springer, Heidelberg

Bucher, M. 2007. Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. New Phytol. 173: 11–26

Budi, S.W., D. van Tuinen, G. Maritinotti, and S. Gianinazzi. 1999. Isolation from the Sorghum bicolor mycorrhizosphere of a bacterium compatible with arbuscular mycorrhiza development and antagonistic towards soil borne fungal pathogens. Appl. Environ. Microbiol. 65:5148-5150.

Buee, M., M. Rossignol, A. Jauneau, R. Ranjeva, G. Be´card. 2000. The pre- symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Mol. Plant Microbe Interact. 13:693-698.

Caetano-Anollés, G., and W.D. Bauer. 1988. Enhanced nodule initiation on alfalfa by wild-type *Rhizobium meliloti* co-inoculated with nod gene mutants and other bacteria. Planta. 174; 3 :385-95.

Carling, D.E., W.G. Reihle, M.F. Brown, D.R. Johnston. 1978. Effects of vesicular- arbuscular mycorrhizal fungus on nitrate reductase and nitrogenase activities in nodulating and non-nodulating soybeans. Phytopathology. 68: 1590–1596.

Carneiro, M.A.C., J.O. Siqueira, F.M.D. Moreira. 2001. Estabelecimento de plantas herba´ceas em solo com contaminac¸a˜o de metais pesados e inoculac¸a˜o de fungos micorr`Izicos arbusculares. Pesqui. Agropecu. Bras. 36: 1443–1452.

Carpenter-Boggs, L., T. E. Loynachan, P.D. Stahl. 1995. Spore germination of Gigaspora margarita stimulated by volatiles of soil-isolated actinomycetes. Soil Biol. Biochem. 27:1445– 1451

Catford, J.G, C. Staehelin, G. Larose, Y. Pich´e, H. Vierheilig. 2006. Systemic suppressed isoflavonoids and their stimulating effects on nodulation and mycorrhization in alfalfa split-root systems. Plant Soil 285: 257–266

Catford, J.G., C. Staehelin, S. Lerat, Y. Pich´e, H. Vierheilig. 2003. Suppression of arbuscular mycorrhizal colonization and nodulation in split-root systems of alfalfa after pre-inoculation and treatment with Nod factors. J. Exp. Bot. 54: 1481–1487

Champawat, R.S., and V.N. Pathak. 1993. Effect of Vesicular-arbuscular mycorrhizal fungi on growth and nutrition uptake of pearl millet. Indian J. Mycol. Plant. Pathol. 23(1):30-34.

Chaturvedi, C., and R. Singh. 1986. Response of chickpea (*Cicer arietinum* L.) to inoculation with *Rhizobium* and VA mycorrhiza. Proc. Natl. Acad. Sci. India Sect B Biol Sci 59: 443–446

Chen, C.Y., M.Q. Gao, J.Y. Liu, and H.Y. Zhu. 2007. Fungal symbiosis in rice requires an ortholog of a legume common symbiosis gene encoding a Ca2+/calmodulin-dependent protein kinase. Plant Physiol. 145:1619-1628.

Chen, C.Y., J.M. Ané, and H.Y. Zhu. 2008. OsIPD3, an ortholog of the *Medicago truncatula* DMI3 interacting protein IPD3, is required for mycorrhizal symbiosis in rice. New Phytol. 180:311-315.

Chen, C.Y., M.Q. Gao, J.Y. Liu, and H.Y. Zhu. 2009. Antiquity and function of CASTOR and POLLUX, the twin ion channel-encoding genes key to the evolution of root symbioses in plants. Plant Physiol. 149:306- 317.

Christensen, H, and I. Jakobsen. 1993. Reduction of bacterial growth by a vesicular–arbuscular mycorrhizal fungus in the rhizosphere of cucumber (*Cucumis sativus* L.). Biol Fertil Soils 15:253–258

Clark, R.B., and S.K. Zeto. 2000. Mineral acquisition by arbuscular mycorrhizal plants. J. Plant Nutr. 23: 867–902.

Cleveland, C.C., A.R. Townsend, D.S. Schimel, H. Fisher, R.W. Howarth, L.O. Hedin, S.S. Perakis, E.F. Latty, J.C. von Fischer, A. Elseroad, and M.F. Wasson. 1999. Global patterns of terrestrial biological nitrogen fixation in natural ecosystems. Global Biogeochem. Cycles. 13: 623-645.

Cochran, W.G. 1950. Estimation of bacterial densities by means of the "most probable number". Biometrics 6:105 116.

Cordier, C., M.C. Lemoine, P. Lemanceau, V. Gianinazzi-Pearson and S. Gianinazzi. 1999. The beneficial rhizosphere: a necessary strategy for microplant production. Acta Horticulturae 530:259–265.

Cuenca, G., and R. Azcón. 1994. Effects of ammonium and nitrate on the growth of vesicular-arbuscular mycorrhizal *Eiythrina poeppigiana* O.I. cook seedlings. Biol. Fertil. Soils 18:249-254.

Curl, E. A., and B. Truelove. 1986. The rhizosphere. New York: Springer-Verlag. p. 288

D'Haeze, W., and M. Holsters. 2002. Nod factor structures, responses, and perception during initiation of nodule development. Glycobiol. 12:79R-105R.

Daei, G., M. Ardakani, F. Rejali, S. Teimuri, and M. Miransari. 2009. Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. J. Plant Physiol. 166:617–625

Daniels, B.A. and J.M. Trappe. 1980. Factors affecting spore germination of the vesicular–arbuscular mycorrhizal fungus, *Glomus epigaeus*. Mycologia 72:457–471

De Boer, W., L.B. Folman, R.C. Summerbell, and L. Boddy. 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol. Rev. 29:795–811.

De Clerck, F., M.J. Singer, and P. Lindert. 2003. A 60-year history of California soil quality using paired samples. Geoderma. 114: 215–230.

De Oliveira, V.L. 1988. Interactions entre les microorganisnies du sol et l 'etablissemeni de la symbiose ectomycorhizienne chez le heter (*Fagus sylvatica L.*) avec *Hebeloma crustuliniforme* et *Paxillus involutus*. Ph.D. thesis of the University of Nancy.

De Oliveira, V.L., and J. Garbaye 1989. Les microorganisms' auxiliaires de retabhssemeni des symbioses ectomycorrbiziennes. Euro. J. Fore. Path. 19:54—64.

Demchenko, K., T. Winzer, J. Stougaard, M. Parniske, and K. Pawlowski. 2004. Distinct roles of *Lotus japonicus* SYMRK and SYM15 in root colonization and arbuscule formation. New Phytol. 163:381-392.

Dickson, S., and P. Kolesik. 1999. Visualisation of mycorrhizal fungal structures and quantification of their surface area and volume using laser scanning confocal microscopy. Mycorrhiza. 9:205-213.

Douds, D.D., and C. Reider. 2003. Inoculation with mycorrhizal fungi increases the yield of green peppers in a high P soil. Biol. Agric. Hortic. 2:91–102

Douds, D.D., G. Nagahashi Jr., P.E. Pfeiffer, W.M. Kayser, and C. Reider. 2005. On-farm production and utilisation of arbuscular mycorrhizal fungus inoculum. Can. J. Plant Sci. 85:15–21.

Drew, E.A., R.S. Murray, S.E. Smith, and I. Jakobsen. 2003. Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. Plant Soil. 251:105–114

Duponnois, R., C. Plenchette, and A.M. Ba. 2001. Growth stimulation of seventeen fallow leguminous plants inoculated with *Glomus aggregatum* in Senegal. Euro. J. Soil Bio. 37:181- 186.

Durall, D.M., J.D. Marshall, M.D. Jones, R. Crawford, and J.M. Trappe. 1994. Morphological changes and photosynthate allocation in ageing *Hebeloma crustuliniforme* and *Laccaria bicolor*, mycorrhizas of *Pinus ponderosa* Douglus. New Phytol. 127:719–724.

Ellis, J.G., D.F. Acton and H.C. Moss. 1970. The Soils of the Rosetown Map Area 72-0 Saskatchewan. Saskatchewan Institute of Pedology Publication S3. Modern Press, Saskatoon

Estaun V., A. Camprubi, and E.J. Joner. 2002. Selecting arbuscular mycorrhizal fungi for field application. In: Mycorrhizal technology in agriculture. Eds. Gianinazzi S., H. Schuepp, J.M. Barea, and K. Haselwandter. Birkhauser Verlag, Berlin. pp. 249-259.

Feddermann, N., Roger R. Finlay, T. Boller, and M. Elfstrand. 2010. Functional diversity in arbuscular mycorrhiza-the role of gene expression, phosphorous nutrition and symbiotic efficiency. Fungal Ecol. 3:1–8

Feldman, F., and Grotkass, C. 2002. Directed inoculum production – shall we be able to design populations of arbuscular mycorrhizal fungi to achieve predictable symbiotic effectiveness? In: Mycorrhiza Technology in Agriculture, from Genes to Bioproducts. S. Gianinazzi, H. Schüepp, J. M. Barea and K. Haselwandter. Eds. Birkhäuser Verlag: Basel, Switzerland .p. 261-296

Feng, G., F.S. Zhang, X.L. Li, C.Y. Tian, C. Tang, and Z. Rengel. 2002. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza 12:185–190.

Fester, T., D. Strack, and B. Hause. 2001. Reorganization of tobacco root plastids during arbuscule development. Planta. 213:864-868.

Fitter, A.H., and Garbaye, J. 1994. Interactions between mycorrhizal fungi and other soil organisms. Plant Soil. 159:123–132.

Francis, R., and D.J. Read. 1995. Mutualism and antagonism in the mycorrhizal symbiosis, with special reference to impacts on plant community structure. Can. J. Bot. 73:1301–1309

Frank, A.B. 1887. Ueber neue Mycorrhiza-formen. Ber Dtsch Bot Ges 5:395–409

Frank, A.B. 1885. Ueber die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. Ber Dtsch Bot Ges 3:128–145 [In English translation.2005. Mycorrhiza]

Franzini, VI., R. Azcón, F. Latanze-Mendes, and R. Aroca. 2010. Interaction between Glomus species and Rhizobium strains affect the nutritional physiology of drought stressed legume hosts. J. Plant Phys. 167:614–619.

Frey-Klett, P., J. Garbaye, and M. Tarkka. 2007. The mycorrhiza helper bacteria revisited. New Phytol. 176:22–36.

Gage, D.J., and W. Margolin. 2000. Hanging by a thread: invasion of legume plants by rhizobia. Curr. Opin. Microbiol 3:613–617.

Galleguillos, C., C. Aguirre, J. M. Barea, and R. Azcón. 2000. Growth promoting effect of two *Sinorhizobium meliloti* strains (a wild type and its genetically modified derivative) on a non-legume plant species in specific interaction with two arbuscular mycorrhizal fungi. Plant Sci. 159:57-63.

Gamalero, E., G. Lingua, G. Berta, and B.R. Glick. 2009. Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can. J. Microbiol. 55:501-514.

Ganry, F., H.G. Diem, and Y.R. Dommergues. 1982. Effect of inoculation with *Glomus mosseae* on nitrogen fixation by field grown soybeans. Plant Soil. 68:321-329.

Garbaye, J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol. 128:197-210.

Gavito, M.E., and L. Varela. 1995. Response of criollo maize to single and mixed-species inocula of arbuscular mycorrhizal fungi. Plant Soil. 176:101–105.

Gavito, M.E., P. Schweiger, and I. Jakobsen. 2003. Phosphorus uptake by arbuscular mycorrhizal hyphae: effect of soil temperature and atmospheric CO_2 enrichment. Global Change Biol. 9:106–116

Gavito, M.E., P.S. Curtis, T.N. Mikkelsen, and I. Jakobsen. 2000. Atmospheric CO_2 and mycorrhiza effects on biomass allocation and nutrient uptake of nodulated pea (*Pisum sativum* L.) plants. J. Exp. Bot. 51:1931-1938.

Geneva, M., G. Zehirov, E. Djonova, N. Kaloyanova, G. Georgiev, and I. Stancheva. 2006. The effect of inoculation of pea plants with mycorrhizal fungi and *Rhizobium* on nitrogen and phosphorus assimilation, Plant Soil Environ. 52:435–440.

Genre, A., and P. Bonfante. 1998. Actin versus tubulin configuration in arbuscule-containing cells from mycorrhizal tobacco roots. New Phytol. 140:745-752.

George, E., H. Marschner, and I. Jakobsen. 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. Crit. Rev. in Biotech. 15:257–270.

Geurts, R., E. Fedorova, and T. Bisseling. 2005. Nod factor signaling genes and their function in the early stages of *Rhizobium* infection. Curr. Opin. Plant Biol. 8:346–352.

Gianinazzi, S., and H. Schüepp. 1994. Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems. ALS Birkhäuser Verlag: Basel, Switzerland.

Gianinazzi, S., H. Schüepp, J. M. Barea, and K. Haselwandter. 2002. Mycorrhizal Technology in Agriculture, from Genes to Bioproducts. Birkhäuser Verlag: Basel, Switzerland.

Gianinazzi-Pearson, V. 1997. Have common plant systems co-evolved in fungal and bacterial root symbioses? In: Biological fixation of Nitrogen for ecology and sustainable agriculture. A. Legocki, H. Bothe and A. Pühler. Eds. Spinger-Verlag: Berlin and Heidelberg, Germany. p. 322-324.

Gianinazzi-Pearson,V. 1996. Plant cell responses to arbuscular mycorrhizal fungi: Getting to the roots of the symbiosis. Plant Cell 8:1871–1883.

Giovannetti, M. 2001. Spore germination and pre-symbiotic mycelial growth. In: Arbuscular Mycorrhizas: Physiology and Function. Y. Kapulnik and D. D. Douds Jr. .Eds. Kluwer Academic Publishers: Dordrecht, The Netherlands. p. 3-18.

Glick, B.R. 1995. The enhancement of plant growth by free living bacteria. Can. J. Microbiol. 41:109-117.

Gosling, P., A. Hodge, G. Goodlass, and G.D. Bending. 2006. Arbuscular mycorrhizal fungi and organic farming. Agric. Ecosyst. Environ. 113:17–35.

Goss, M.J., A. de Varennes. 2002. Soil disturbance reduces the efficacy of mycorrhizal associations for early soybean growth and N_2 fixation. Soil Biol. and Biochem. 34:1167–1173.

Gough, C., J. Cullimore. 2011. Lipo-chitooligosaccharide signaling in endosymbiotic plant-microbe interactions. Mol. Plant Microbe Interact. 24:867-78

Govindarajulu, M., P.E. Pfeffer, H. Jin, J. Abubaker, and D.D. Douds, J.W. Allen, H. Bucking, P.J. Lammers, Y. Shachar-Hill. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature. 435:819-823.

Grace, E.J., O. Cotsaftis, M. Tester, F.A. Smith, S.E. Smith. 2009. Arbuscular mycorrhizal inhibition of growth in barley cannot be attributed to extent of colonization, fungal phosphorus uptake or effects on expression of plant phosphate transporter genes. New Phytol. 181: 938–949

Graham, J.H., 2000. Assessing cost of arbuscular mycorrhizal symbiosis in agroecosystems. In: Current Advances in Mycorrhizal Research. Podila, G.K., and D.D. Douds, Jr. Eds. APS Press: St. Paul, NM. p.127–140.

Grayston, S.J., S. Wang, C.D. Campbell, and A.C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Bio. and Biochem. 30:369 – 378.

Gryndler, M., H. Hrselova, and D. Striteska.2000. Effect of soil bacteria on hyphal growth of the arbuscular mycorrhizal fungus *Glomus claroideum*. Folia. Microbiol. 45:545–551

Gucwa-Przepióra, E., and K. Turnau. 2001. Arbuscular mycorrhiza and plant succession in the zinc smelter spoil heap in Katowice- Welnowiec. Acta. Soc. Bot. Pol. 70:153–158

Hamel, C., Y. Dalpe´, C. Lapierre, R.R. Simard, and D.L. Smith. 1994. Composition of the vesicular-arbuscular mycorrhizal fungi population in an old meadow as affected by pH, phosphorus and soil disturbance. Agric. Ecosyst. Environ. 49:223–231.

Hamel, C., Y. Dalpe´, V. Furlan, and S. Parent. 1997. Indigenous populations of arbuscular mycorrhizal fungi and soil aggregate stability are major determinants of leek (*Allium porrum* L.) response to inoculation with *Glomus intraradices* Schenck & Smith or *Glomus versiforme* (Karsten) Berch. Mycorrhiza. 7:187–196.

Hangs, R.D, Schoenau, J.J., and Van Rees, K.C.J. 2012. A Novel Technique for Rapidly Separating Willow Roots from Clay Soil. In Proc. Soils and Crops Workshop. University of Saskatchewan. Saskatoon, SK.

Harinikumar, K.M., D.J. and Bagyaraj. 1996. Persistence of introduced *Glomus intraradices* in the field as influenced by repeated inoculation and cropping system. Biol. Fertil. Soil 21:184–188.

Harley, J. L. 1971. Fungi in ecosystems. J. Appl. Ecol. 8:627-642.

Harley, J. L., and S. E. Smith, 1983. Mycorrhizal symbioses. Academic Press: London. p. 483.

Harris, D., R. S. Pacovsky, and E. A. Paul. 1985. Carbon economy of soybean-*Rhizobium*-*Glomus* associations. New Phytol. 101:427-440.

Harrison, M.J., G.R. Dewbre, and J.Y. Liu. 2002. A phosphate transporter from *Medicago truncatula* involved in the acquisiton of phosphate released by arbuscular mycorrhizal fungi. Plant Cell. 14:2413-2429.

Haselwandter, K. 1997. Soil micro-organisms, mycorrhiza, and restoration ecology. In: Urban´ska, K., N.R. Webb, P.J. Edwards. Eds. Restoration ecology and sustainable development. Cambridge University Press: Cambridge. p. 65–80.

Hawkins, H.J., A. Johansen, and E. George. 2000. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. Plant and Soil 226: 275–285.

Hayman, D. S. 1986. Mycorrhizae of nitrogen-fixing legumes. MIRCEN Journal. 2:121-145.

Heath, K. D., A.J. Stock, and J R. Stinchcombe. 2010. Mutualism variation in the nodulation response to nitrate. J. Evol. Biol. 23:1-7.

Herridge, D.F., Peoples, M.B, Boddey, R,M. 2008. Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311:1–18

Hiltner, L.Ü. 1904. Ber neuere Erfahrungen und Probleme auf dem Gebiet der BerÜ cksichtigung der GrÜ ndÜ ngung und Brache. Arb Deutsch Landwirt Ges. 98:59-78.

Hinsinger, P., G.R. Gobran, P.J. Gregory, and W.W. Wenzel. 2005. Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. New Phytol. 168:293-303.

Hirsch, A.M., 1992. Tansley Review no. 40: Developmental biology of legume nodulation. New Phytologist 122, 211-237.

Hinkelmann, K. and Oscar Kempthorne. 2008. Design and Analysis of Experiments, Volume I and II, Second ed. Wiley. ISBN 978-0-470-38551-7.

Hoagland, D.R. and D.I. Arnon. 1938. The water culture method for growing plants without soil. Circ. Calif. Agr. Exp. Stn. no. 347.

Hodge, A. 2003. Plant nitrogen capture from organic matter as affected by spatial dispersion, interspecific competition and mycorrhizal colonisation. New Phytologist 157: 303–314.

Hodge, A. 2000. Microbial ecology of the arbuscular mycorrhizal. FEMS Microbiol. Ecol. 32:91-96.

Hodge, A., C.D. Campbell, and A.H. Fitter 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature. 413:297-299.

Ianson, D.C., and R.G. Linderman. 1993. Variation in the response of nodulating pigeonpea (*Cajanus cajan*) to different isolates of mycorrhizal fungi. Symbiosis 15:105–119

Ibibijen, J., S. Urquiaga, M. Ismaili, B.J. Alves, and R.M. Boddey. 1996. Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition and nitrogen fixation of three varieties of common beans (*Phaseolus vulgaris*). New Phytol. 134:353–360.

Idris, A., N. Labuschagne, and L. Korsten. 2009. Efficacy of rhizobacteria for growth promotion in sorghum under greenhouse conditions and selected modes of action studies. J. Agric. Sci. 147:17-30.

Islam, R., and A. Ayanaba. 1981. Effect of seed inoculation and pre-infecting cowpea (*Vigna unguiculata*) with *Glomus mosseae* on growth and seed yield of the plants under field conditions. Plant Soil, 61: 341-350

Izaguirre-Mayoral, M.L., O. Carballo, L. Carreno and M.G. de Mejia. 2000. Effects of arbuscular mycorrhizal inoculation on growth, yield, nitrogen, and phosphorus nutrition of nodulating bean varieties in two soil substrates of contrasting fertility. J. Plant Nutr. 23:1117-1133.

Jacquot, E., D. van Tuinen, S. Gianinazzi, and V. Gianinazzi-Pearson. 2000. Monitoring species of arbuscular mycorrhizal fungi in planta and in soil by nested PCR: application to the study of the impact of sewage sludge. Plant Soil. 226:179–188

Jacquot-Plumey, E., D. van Tuinen, O. Chatagnier, S. Gianinazzi, and V. Gianinazzi-Pearson. 2001. 25S rDNA-based molecular monitoring of glomalean fungi in sewage sludge-treated field plots. Environ. Microbiol. 3:525–531

Jakobsen, I., and Rosendahl, L., 1990. Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber roots. New Phytol. 115:77–83.

Jasper, D.A., A.D. Robson, and L.K. Abbott. 1979. Phosphorus and the formation of vesicular arbuscular mycorrhizas. Soil Biol. Biochem. 11:501–505.

Javot, H., R.V. Penmetsa, N. Terzaghi, D.R. Cook, and M.J. Harrison. 2007. A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. Proceedings of the National Academy of Sciences, U.S.A. 104:1720-1725.

Jeffries, P, and M.J. Barea. 2001. Arbuscular mycorrhiza: a key component of sustainable plant–soil ecosystems. In: The Mycota. Vol IX: fungal associations. Hock B. Ed. Springer: Berlin Heidelberg New York. p. 95–113

Jeffries, P., A. Craven-Griffiths, J.M. Barea, Y. Levy, and J.C. Dodd. 2002. Application of arbuscular mycorrhizal fungi in the revegetation of desertified Mediterranean ecosystem. In: Gianinazzi, S., H. Schüepp, J. M. Barea and K. Haselwandter. Eds. Mycorrhiza Technology in Agriculture, from Genes to Bioproducts. ALS, Birkhäuser, Verlag: Heidelberg, Germany. p. 151-174.

Jensen, A., and I. Jakobsen. 1980. The occurance of vesicular-arbuscular mycorrhiza in barley and wheat grown in some Danish soils with different fertiliser treatments. Plant Soil 55:403–414.

Jia, Y., V.M. Gray, and C.J. Straker. 2004. The Influence of *Rhizobium* and arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *Vicia faba*. Ann. Bot. 94:251- 258.

Johansen, A., I. Jakobsen, and E.S. Jensen. 1992. Hyphal transport of 15N-labelled nitrogen by a vesicular-arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. New Phytol. 122:281-288.

Johansen, A., I. Jakobsen, and E.S. Jensen. 1994. Hyphal N transport by a vesicular arbuscular mycorrhizal fungus associated with cucumber grown at three nitrogen levels. Plant Soil 160:1-9.

Johansson, J.F., L.R. Paul, and R.D. Finlay. 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiol. Ecol. 48:1–13.

Jones, M.D., and S.E. Smith. 2004. Exploring functional definitions of mycorrhizas: are mycorrhizas always mutualisms? Can. J. Bot. 82:1089–1109.

Kahiluoto, H., and M. Vestberg. 1998. The effect of arbuscular mycorrhiza on biomass production and phosphorus uptake from sparingly soluble sources by leek (*Allium porrum* L.) in Finnish field soils. Biol. Agric. Hortic. 16:65–85

Kapoor, R., B. Giri, and K.G. Mukerji. 2002. Mycorrhization of coriander (*Coriandrum sativum* L.) to enhance the concentration and quality of essential oil. J. Sci. Food Agr. 82:339–342.

Kapoor, R., B. Giri, and K.G. Mukerji. 2004. Improved growth and essential oil yield and quality in *Foeniculum vulgare* mill on mycorrhizal inoculation supplemented with P-fertilizer. Biores. Technol. 93:307–311.

Kapulnik, Y., and D.D. Douds, Jr. 2000. Arbuscular Mycorrhizas: Physiology and Function. Kluwer Academic Publishers: Dordrecht, The Netherlands.

Karandashov, V, and M. Bucher. 2005. Symbiotic phosphate transport in arbuscular mycorrhizas. Trends in Plant Science. 10:22–29.

Karpenstein-Machan, M., and R. Stuelpnagel. 2000. Biomass yield and nitrogen fixation of legumes monocropped and intercropped with rye and rotation effects on a subsequent maize crop. Plant and Soil 218:215–232.

Kaschuk G., P.A. Leffelaar, K.E. Giller, O. Alberton, M. Hungria and T.W. Kuyper. 2010. Responses of legumes to rhizobia and arbuscular mycorrhizal fungi: A meta-analysis of potential photosynthate limitation of symbioses. Soil Biol. and Biochem. 42:125-127.

Kawai, Y., and Y. Yamamoto. 1986. Increase in the formation and nitrogen fixation of soybean nodules by vesicular-arbuscular mycorrhiza. Plant Cell Physiol. 27:399–405

Kennedy, A. C. 1998. The rhizosphere and spermosphere. In: Principles and Applications of Soil Microbiology. Sylvia, D. M., J. J. Fuhrmann, P. G. Hartel and D. A. Zuberer. Eds. Prentice Hall :Upper Saddle River, NJ. p. 389-407

Khalil, S., T.E. Loynachan, and H.S. McNabb. 1992. Colonization of soybean by mycorrhizal fungi and spore populations in Iowa soils. Agron. J. 84:832–836

Kiers, E. T., R. A. Rousseau, S. A. West, and R. F. Denison. 2003. Host sanctions and the legume rhizobium mutualism. Nature. 425:78–81.

Kistner, C., T. Winzer, A. Pitzschke, L. Mulder, S. Sato, T, Kaneko, S. Tabata, N. Sandal, J. Stougaard, K.J. Webb et al. 2005. Seven Lotus japonicus genes required for transcriptional reprogramming of the root during fungal and bacterial symbiosis. Plant Cell. 17:2217-2229.

Klironomos, J.N. 2003. Variation in plant response to native and exoticarbuscular mycorrhizal fungi. Ecology, 84:2292–2301.

Klironomos, J.N., and M.M. Hart. 2002. Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. Mycorrhiza 12:181–184.

Kloepper, J. W. 1994. Plant growth-promoting rhizobacteria .other systems.. In: *Azospirillum* / plant associations. Okon Y., Ed. CRC Press: Boca Raton, FL. p. 111-118.

Kloepper, J.W.1996. Host specificity in microbe–microbe interactions. Bioscience 46:406–409

Koch, K.E., and C.R. Johnson. 1984. Photosynthate partitioning in slit root seedlings with mycorrhizal root systems. Plant Physio. 75:26–30.

Kogelmann, W.J., H.S. Lin, R.B. Bryant, D.B. Beege, A.M. Wolf, and G.W. Petersen. 2004. A statewide assessment of the impacts of phosphorus-index implementation in Pennsylvania. J. Soil Water Conserv. 59:9–18

Koide, R.T and Z. Kabir. 2000. Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyze organic phosphate. New Phytol. 148:511-517.

Koide, R.T. and B. Mosse. 2004. A history of research on arbuscular mycorrhiza. Mycorrhiza. 14:145-163.

Kosuta, S., M. Chabaud, G. Lougnon, C. Gough, J. De´narie´, D.G. Barker, and G. Be´card. 2003. A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific MtENOD11 expression in roots of Medicago truncatula. Plant Physiol. 131:952-962.

Kouchi, H., H.Imaizumi-Anraku, M.Hayashi, T. Hakoyama, T. Nakagawa, Y. Umehara, N. Suganuma, and M. Kawaguchi. 2010. How many peas in a pod? Legume genes responsible for mutualistic symbioses underground. Plant Cell. Physiol. 51:1381–1397

Krüger, M., C. Krüger, C. Walker, H. Stockinger, and A. Schüßler. 2012. Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol. 193:970-987.

Kucey, R. M. N., and E. A. Paul. 1982. Carbon flow in photosynthesis, and N_2 fixation in mycorrhizal and nodulated faba beans (*Vicia faba* L.) Soil Biol. Biochem. 14:407-412.

Kucey, R. M. N., and E. A. Paul. Carbon flow in plant microbial associations. Science. 213:473–474.

Kumar, A., Nivedita and R.S. Upadhyaya. 1999. VA mycorrhizae and revegetation of coal mine spoils: a review.Tropical Ecology 40: 1-10.

Lima, P. T., V. G. Faria, P. Patraquim, A. C. Ramos, J. A. Feijó, and E. Sucena. 2009. Plant-microbe symbioses: new insights into common roots. BioEssays : news and reviews in molecular, cellular and developmental biology, 31:1233-44.

Linderman, R.G. 1988. Mycorrhizal interactions with the rhizosphere microflora-the mycorrhizosphere effect. Phytopathology 78:366– 371

Linderman, R.G. 1992. Vesicular-arbuscular mycorrhizae and soil microbial interactions. In: Bethlenfalvay G.J., and R.G. Linderman. Eds.Mycorrhizae in sustainable agriculture. ASA special publication ASA: Madison, Wis. p.1–26

Linderman, R.G. 2000. Effects of mycorrhizas on plant tolerance to diseases. In: Arbuscular mycorrhizas: Physiology and function. Kapulnik Y., and D.D.J. Douds. Eds. Kluwer Academic Publishers: Dordrecht. p. 345–365

Liu, A., C. Hamel, A. Elmi, C. Costa, B. Ma, and D.L. Smith. 2002. Concentrations of K, Ca and Mg in maize colonized by arbuscular mycorrhizal fungi under field conditions. Can. J. Soil Sci. 82:271–278.

Lodwig, E.M., A.H.F. Hosie, A. Bourdis, K. Findlay, D. Allaway, R. Karunakaran, J.A. Downie, and P.S. Poole. 2003. Amino-acid cycling drives nitrogen fixation in the legume-Rhizobium symbiosis. Nature. 4: 722-726.

Louis, I., and G. Lim. 1988. Differential growth and mycorrhizal colonization of soybean to inoculation with two isolates of *Glomus clarum* in soils of different P availability. Plant and Soil 112:37-43

Lovelock, C.E., S.F. Wright, D.A. Clark, and R.W. Ruess. 2004. Soil stocks of glomalin produced by arbuscular mycorrhizal fungi across a tropical rain forest landscape. J. Ecol. 92:278–287.

Lovera, M., and G. Cuenca. 1996. Arbuscular mycorrhizal infection in Cyperaceae and Gramineae from natural disturbed and restored savannas in La Gran Sabana, Venezuela. Mycorrhiza 6:111– 118

Ma¨der, P., S. Edenhofer, T. Boller, A. Wiemken, and U. Niggli. 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input organic biological and high-input conventional farming systems in a crop rotation. Biol. Fertil. Soil. 31:150–156.

Maia, L.C., and J.W. Kimbrough. 1998. Ultrastructural studies of spores and hypha of a Glomus species. Intl. J. Plant Sci. 159:581–589.

Maillet, F., V. Poinsot, O. Andre, V. Puech-Pages, A. Haouy, and M. Gueunier. 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature. p. 469.

Marcel, G.A., V.D. Heijden, R.D. Bardgett, and N.M.V. Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett. 11:296–310.

Mardukhi, B., F. Rejali, G. Daei, M.R. Ardakani, M.J. Malakouti, and M. Miransari. 2011. Arbuscular mycorrhizas enhance nutrient uptake in different wheat genotypes at high salinity levels under field and greenhouse conditions. CR Biol. 334:564–571

Marschner, H. 1995 Mineral Nutrition in Plants. Academic. 2nd Edition. San Diego, CA (USA).p.889.

Marulanda, A., J.M. Barea, and R. Azcon. 2006. An indigenous drought tolerant strain of *Glomus intraradices* associated with a native bacterium improves water transport and root development in *Retama sphaerocarpa*. Microb. Ecol. 52:670–678

Matusova, R., K. Rani, F.W.A. Verstappen, M.C.R. Franssen, M.H. Beale, and H.J. Bouwmeester. 2005. The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. Plant Physiol. 139:920–934

Mayo, K., R.E. Davis, and J. Motta. 1986. Stimulation of germination of spores of *Glomus versiforme* by spore-associated bacteria. Mycologia. 78:426-431.

Mead, R., Robert N. Currow and Anne M. Hasted. 2003. Statistical Methods in Agriculture and Experimental Biology, Third Edition, CRC press, U.S.A.

Mehdi, Z., S.R. Nahid, H.A. Alikhani and A. Nasser. 2006. Responses of lentil to co-inoculation with phosphate-solubilizing rhizobial strains and arbuscular mycorrhizal fungi. J. Plant Nutr. 29:1509-1522.

Meyer, J.R., and R.G. Linderman. 1986. Response of subterranean clover to dual inoculation with vesicular-arbuscular fungi and a plant growth-promoting bacterium, *Pseudomonas putida*. Soil Biol. Biochem. 18:185–190

Mikanovμ, O., J. Kubμt, I. Mikhailovskaya, I. Vörös, and B. Biró. 2001. Influence of heavy metal pollution on some soil-biological parameters in the alluvium of the Litavka River. Rostl Vyroba. 47:117–122

Miller, R.M., and J.D. Jastrow. 2000. Mycorrhizal fungi influence soil structure. In: Kapulnik, Y., and D.D. Douds. Eds. Arbuscular mycorrhizas: physiology and function. Kluwer Academic: Dordrecht, The Netherlands. p. 3–18

Moawad, A. M., and L. P. G. Vlek. 1997. Potential contribution of vesicular.-arbuscular mycorrhiza to nutrient efficient crops. In: Proceedings of the BTIG Workshop on Oil Palm Improvement Through Biotechnology. Ministry of Agriculture: Bogor, Indonesia. p. 48-58.

Mohammad, A., B. Mitra, and A.G. Khan. 2004. Effects of sheared-root inoculum of Glomus intraradices on wheat grown at different phosphorus levels in the field. Agric. Ecosyst. Environ. 103:245–249.

Mohammad, M.J., H.I. Malkawi, and R. Shibli. 2003. Effects of mycorrhizal fungi and phosphorus fertilization on growth and nutrient uptake of barley grown on soils with different levels of salts. J. Plant Nutr. 26:125–137

Mortimer, P.E., M.A. Perez-Fernandez, and A.J. Valentine. 2008. The role of arbuscular mycorrhizal colonization in the carbon and nitrogen economy of the tripartite symbiosis with nodulated *Phaseolus vulgaris*. Soil Biol. Biochem. 40:1019–1027

Mosse, B. 1953. Fructifications associated with mycorrhizal strawberry roots. Nature 171:974

Mosse, B. 1959. Observations on the extramatrical mycelium of vesicular-arbuscular endophytes. Transactions of the British Mycological Society. 42: 439–448.

Mosse, B. 1986. Mycorrhiza in a sustainable agriculture. Biol. Agric., 3, 191-209.

Napoli, C., A. Mello, and P. Bonfante. 2008. Dissecting the rhizosphere complexity: The truffle-ground study case. Rendiconti Lincei. 19:241-259.

Nautiyal, C.S., P.S. Chauhan, S.M. DasGupta, K. Seem, A. Varma, W.J. Staddon. 2010. Tripartite interactions among *Paenibacillus lentimorbus* NRRL B-30488, *Piriformospora indica* DSM 11827, and *Cicer arietinum* L.W. J. Microbiol. Biotech. 26:1393-1399.

Nelson, L.M. 2004. Plant growth promoting rhizobacteria (PGPR): prospects for new inoculants. 2004 Plant Management Network, online doi:10.1094/CM-2004-0301-05-RV

Nogueira, M.A., G.C. Magalhães, and E.J.B.N. Cardoso. 2004. Manganese toxicity in mycorrhizal and phosphorus-fertilized soybean plants. J. Plant Nutr. 27:141–156

Nwoko, H., and N. Sanginga. 1999. Dependence of promiscuous soybean and herbaceous legumes on arbuscular mycorrhizal fungi and their response to bradyrhizobial inoculation in low P soils. Applied Soil Ecology 13:251-258.

Oldroyd, G.E.D, M.J. Harrison, M. Udvardi. 2005. Peace talks and trade deals: keys to long-term harmony in legume-microbe symbioses. Plant Physiol. 137:1205–1210

Olivera, M., N. Tejera, C. Iribarne, A. Ocana, and C. Lluch. 2004. Growth, nitrogen fixation and ammonium assimilation in common bean *Phaseolus vulgaris* effect of phosphorous. Physiologia Plantarum 121:498–505.

Olsson, P.A., I. Thingstrup, I. Jakobsen, and E. Ba˚a˚th. 1999. Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field. Soil Biol. Biochem. 31:1879–1887.

Orłowska, E., S. Zubek, A. Jurkiewicz, G. Szarek-Lukaszewska, and K. Turnau. 2002. Influence of restoration on arbuscular mycorrhiza of (*Biscutella laevigata* L.) Brassicaceae and (*Plantago lanceolata* L) Plantaginaceae from calamine spoil mounds. Mycorrhiza 12:153–159

Pacovsky, R.S., G. Fuller, and A.E. Stafford. 1986. Nutrient and growth interactions in soybeans colonized with *Glomus fasciculatum* and *Rhizobium japonicum*. Plant Soil. 92:37–45

Parniske, M. 2000. Intracellular accommodation of microbes by plants: a common developmental program for symbiosis and disease? Curr. Opin. Plant Biol. 3:320–328

Parniske, M. 2004 Molecular genetics of the arbuscular mycorrhizal symbiosis. Curr. Opin. Plant Biol. 7:414-421.

Parniske, M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat. Rev. Microbiol. 6:763–775

Paula, M.A., S. Urquiaga, J.O. Siqueira, and J. Döbereiner. 1992. Synergistic effects of vesicular-mycorrhizal fungi and diazotrophic bacteria on nutrition and growth of sweet potato (*Ipomea batatas*). Biol. Fert. Soils 14:61–66.

Pearson, J.N., L.K. Abbott, and D.A. Jasper. 1993. Mediation of competition between two colonizing VA mycorrhizal fungi by the host plant. New Phytol. 123:93–98

Phillips, R.P., and T.J. Fahey. 2008. The influence of soil fertility on rhizosphere effects in northern hardwood forest soils. Soil Sci. Soc. Am. J. 72:453-461.

Popp, C., J. Folgmann, and T. Ott. 2014. How to suppress nodule organogenesis during arbuscular mycorrhiza despite activation of the `common sym´ signaling pathway? In: Proceedings of the XVI International Congress on Molecular Plant-Microbe Interactions, 6-10 July 2014, Rhodes, Greece.

Porter, W.N. 1979. The "most probable number" method for enumerating infective propagules of vesicular–arbuscular mycorrhizal fungi in soil. Austral. J. Soil Res. 17:515–519.

Pozo, M.J., C. Cordier, E. Dumas-Gaudot, S. Gianinazzi, and J.M. Barea, C. Azco´n-Aguilar, 2002. Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. J. Exp. Bot. 53:525–534.

Preliminary Soil Map and Report. 1970. The Soils of the Swift Current Area. Saskatchewan Soil Survey. Saskatchewan Institute of Pedology, University of Saskatchewan.

Preliminary Soil Map and Report. 1985. Rural Municipality of Whiska Creek, Saskatchewan Soil Survey. Saskatchewan Institute of Pedology, University of Saskatchewan.

Preliminary Soil Map and Report. 1989. Rural Municipality of Whiska Creek, Saskatchewan Soil Survey. Saskatchewan Institute of Pedology, University of Saskatchewan.

Provorov N. A., A. Y. Borisov, and I. A. Tikhonovich. 2002. Developmental genetics and evolution of symbiotic structures in N_2-fixing nodules and arbuscular mycorrhiza. J. Theor. Biol. 214:215-232.

Provorov, N.A., and I.A. Tikhonovich. 2003. Genetic resources for improving nitrogen fixation in legume–rhizobia symbioses. Genetic Research in Crop Evolution 359:907–918.

Rambelli, A. 1973. The rhizosphere of mycorrhizae. In: Ectomycorrhizae: their ecology and physiology. Marks, A.C., and T.T. Kozlowski. Eds. Academic Press: London. p. 229-249.

Rao, N.S.S., K.V.B.R. Tilak, and C.S. Singh. 1986. Dual inoculation with Rhizobium sp. and *Glomus fasciculatum* enhances nodulation, yield and nitrogen fixation in chickpea (*Cicera rietinum* Linn) Plant and Soi1. 95(3):51-359.

Rausch, C., P. Daram, S. Brunner, J. Jansa, M. Laloi, G. Leggewie, N. Amrhein, and M. Bucher. 2001. A phosphate transporter expressed in arbuscule-containing cells in potato. Nature. 414:462-466.

Read, D.J., and J. Perez-Moreno. 2003. Mycorrhizas and nutrient cycling in ecosystems-a journey towards relevance? New Phytol. 157:475–492.

Read. D. J. 1987. Non-nutritional effects of mycorrhizal infection. In: Gianinazzi-Pearson. V., and S. Gianinazzi. Eds. Physiological and Genetical Aspects of Mycorrhizae. Institut National de la Recherche Agronomique: Paris, France. p. 169-176

Redecker D., P. von Berswordt-Wallrabe, and D.P. Beck. 1997. Werner, Influence of inoculation with arbuscular mycorrhizal fungi on stable isotopes of nitrogen in *Phaseolus vulgaris*, Biol. Fertil. Soils. 24:344–346.

Redecker, D., J. B. Morton, and T. D. Bruns. 2000. Ancestral lineages of arbuscular mycorrhizal fungi (*Glomales*). Molecular Phylogenetics and Evolution. 14:276-284.

Reinhardt, D. 2007. Programming good relations - development of the arbuscular mycorrhizal symbiosis. Curr Opin Plant Biol 10:98 – 105

Requena, N., E. Pérez-Solís, C. Azcón-Aguilar, P. Jeffries and J.M. Barea, 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Appl. Environ. Microbiol. 67:495-498.

Richardson, A.E., J.M. Barea, A.M. McNeill, and C. Prigent-Combaret. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil. 321:305-339.

Riely, B.K., J.M. Ane, R.V. Penmetsa, D.R. Cook. 2004. Genetic and genomic analysis in model legumes bring Nod-factor signaling to center stage. Curr. Op. Plant Biol. 7:408-413.

Rillig, M.C., 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. Can. J. Soil Sci. 84:355–363

Rillig, M.C., and D.L. Mummey. 2006. Mycorrhizas and soil structure. New Phytol. 171:41–53.

Rilling, M.C., E.R. Lutgen, P.W. Ramsey, J.N. Klironomos, and J.E. Gannon. 2005 . Microbiota accompanying different arbuscular mycorrhizal fungal isolates influence soil aggregation. Pedobiologis. 49:251-259.

Rufyikiri G., S. Declerck, J. Dufey and B. Delvaux 2000. Arbuscular mycorrhizal fungi might alleviate aluminium toxicity in banana plants. New Phytologist, 148:343-352.

Ruiz-Lozano, J. M., H. Roussel, S. Gianinazzi, and V. Gianinazzi-Pearson. 1999. Defense genes are differentially induced by a mycorrhizal fungus and *Rhizobium* sp. in wild-type and symbiosis- defective pea genotypes. Mol. Plant-Microbe Interact. 12:976-984.

Ruiz-Lozano, J.M., and R. Azcon. 1993. Specificity and functional compatibility of VA mycorrhizal endophytes in association with *Bradyrhizobium* strains in *Cicer arietinum*. Symbiosis. 15:217–226.

Ryan, M.H., and J.F.Angus. 2003. Arbuscular mycorrhizae in wheat and field pea crops on a low P soil increased Zn-uptake but no increase in P uptake or yield. Plant Soil. 250:225–239.

Ryan, M.H., R.M. Norton, J.A. Kirkegaard, K.M. McCormick, S.E. Knights, and J.F. Angus. 2002). Increasing mycorrhizal colonisation does not improve growth and nutrition of wheat on vertisols in south-eastern Australia. J. Agric. Res. 53(10):1173-1181.

Rydlová, J., D. Püschel, R. Sudová, M. Gryndle, O. Mikanová, and M. Vosátka. 2011. Interaction of arbuscular mycorrhizal fungi and rhizobia: Effects on flax yield in spoil-bank clay. J.Plant Nutr. Soil Sci.174:128-134.

Sa, T.M., and D.W. Israel. 1991. Energy status and function of phosphorous-deficient soybean nodules. Plant Physiology 97:928–935.

Sainz, M.J., M.T. Taboada-Castro, and A. Vilarino. 1998. Growth, mineral nutrition and mycorrhizal colonization of red clover and cucumber plants grown in a soil amended with composted urban wastes. Plant Soil. 205:85–92.

Sardans, J., J. Peñuelas, and M. Estiarte. 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. App. Soil Ecol. 39:223-235.

Sarmiento, G., M.P. da Silva, M.E. Naranjo, and M. Pinillos. 2006. Nitrogen and phosphorus as limiting factors for growth and primary production in a flooded savanna in the Venezuelan Llanos. J. Trop. Ecol. 22:203-212.

Saskatchewan Pulse Growers. 2000. Pulse Production Manual., 2nd Ed. Saskatoon, SK.

Saxena, A.K., S.K. Rathi, and K.V.B.R. Tilak. 1997. Differential effect of various endomycorrhizal fungi on nodulating ability of green gram by *Bradyrhizobium sp.* (*Vigna*) strain S24. Biol Fertil. Soils. 24:175–178

Scheublin, T.R., K.P. Ridgway, P.W. Young, and M.G.A. van der Heijden. 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Appl. Environ. Microbiol. 70:6240–6246

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson et al. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Envi. Micro. 75:7537-7541.

Schnepf, A., D. Leitner, S. Klepsch, S. Pellerin, A. Mollier. 2011. Modelling phosphorus dynamics in the soil-plant system. In: Bu¨nemann, E.K., A. Obserson, and E. Frossard. Eds. Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling. Springer: Heidelberg. p. 113–133

Schoeneberger, M.M., R.J. Volk, and C.B. Davey. 1989. Factors influencing early performance of leguminous plants in forest soils. Soil Sci. Soc. Am. J. 53:1429–1434

Schubert, A., and D.S. Hayman. 1986. Plant growth responses to vesicular-arbuscular mycorrhiza. XVI. Effectiveness of different endophytes at different levels of soil phosphate. New Phyto.103:79-90.

Schulze, J., 2004. How are nitrogen fixation rates regulated in legumes? J. Plant Nutri. Soil Sc. 167:125–137.

Schußler, A., D. Schwarzott, and C. Walker. 2001. A new fungal phylum, the Glomeromycota, phylogeny and evolution. Mycol. Res. 105:1413–1421

Secilia, J., and D.J. Bagyaraj. 1988. Fungi associated with pot cultures of vesiculararbuscular mycorrhizas. Trans. Br. Mycol. Soc. 91:117-1 19.

Seneviratne, G., R.M.M.S. Thilakaratne, A.P.D.A. Jayasekara, K.A.C.N. Seneviratne, K.R.E. Padmathilake, and M.S.D.L. De Silva. 2009. Developing beneficial microbial biofilms on roots of non-legumes: a novel biofertilizing technique. In: Microbial strategy for crop improvement. Khan et al. Eds. Springer-Verlag: Berlin. p. 51–61

Shaw, E.J., and R.G. Burns. 2006. Enzyme activities profiles and soil quality. In: Microbiological Methods for Assessing Soil Quality. Bloem, J., D.W. Hopkins, A. Benedetti. Eds. CAB International: Wallingford, UK. p. 158-171.

Sheng, P.P., R.J. Liu, and M. Li. 2012. Inoculation with an arbuscular mycorrhizal fungus and intercropping with epper can improve soil quality and watermelon crop performance in a system previously managed by monoculture. Am-Euras. J. Agri. Env. Sci. 12(11):1462-1468.

Siqueira, J.O., E. Pouyu´, and F.M.S. Moreira. 1999. Micorrizas arbusculares no crescimento po´ s-transplantio de mudas de a´ rvores em solo com excesso de metais pesados. Rev. Bras. Ci. Solo. 23:569–580.

Smith, F.A., E.J.Grace, and S.E. Smith. 2009. More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses. New Phytol. 182:347–358

Smith, S. E., and D. J. Read. 2008. Mycorrhizal Symbiosis. Academic Press, London.

Smith, S.E. 1980. Mycorrhizas of autotrophic higher plants. Biol. Rev.55:475-510.

Smith, S.E., and F.A. Smith. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystems scales. Annu. Rev. Plant Biol. 63:227–250

Smith, S.E., V. Gianinazzi-Pearson, R. Koide, and J.W.G. Caimey. 1994. Nutrient transport in mycorrhizas:structure, physiology and consequences for efficiency of the symbiosis. In: Management of Mycorrhizas in Agriculture. Robson, A.D., L.K. Abbott and N. Malajczuk. Eds. Hort. and Forestry. Kluwer: Dordmcht. p. 103-l 13.

Snellgrove, R.C., W.E. Splittstoesser, D.P. Stribley, and P.B. Tinker. 1982. The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular- arbuscular mycorrhizas. New Phytologist. 92:75–87.

Soil Classification Working Group. 1998. The Canadian System of Soil Classification, 3rd ed. Agriculture and Agri-Food Canada Publication 1646.

Sprent, J. I. 1994. Evolution and diversity in the legume-*Rhizobium* symbiosis: Chaos theory? Plant Soil. 161:1-10.

Srivastava, A.K., S. Singh, R.A. Marathe. 2002. Organic citrus, soil fertility and plant nutrition. J. Sustain. Agric. 19:5-29.

Staff, Saskatchewan Soil Survey. 1989. Preliminary Soil Map and Report of Rural Municipality of Whiska Creek No. 106. Saskatchewan Institute of Pedology, University of Saskatchewan. Agriculture Canada, ERDA.

Staff, Saskatchewan Soil Survey. 1995. The Soils of Sasman Rural Municipality No. 336, Saskatchewan, Saskatchewan Centre for Soil Research Publication SM336.

Tajini, F., M. Trabelsi, and J. Drevon. 2012. Combined inoculation with *Glomus intraradices* and *Rhizobium tropici* CIAT899 increases phosphorus use efficiency for symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris* L.). Saudi J. Biol. Sci. 2012 19(2): 157–163.

Tavasolee, A., N. Aliasgharzad, G. R. Salehi, M. Mardi, A. Asgharzadeh, and S. Akbarivala. 2011. Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on fungal occupancy in chickpea root and nodule determined by real-time PCR. Current Microbiology 63(2):1-8.

Thiagarajan, T. R., R. N. Ames, and M. H. 1992. Ahmad. Response of cowpea (*Vigna unguiculata*) to inoculation with co-selected vesicular–arbuscular mycorrhizal fungi and *Rhizobium* strains in field trials. Canadian Journal of Microbiology. 38: 573-576.

Thomas, R.L., R.W. Sheard, and J.R. Moyer. 1967. Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion. Agron. J. 59:240–243

Thompson, J.N. 1982. Interaction and coevolution, John Wiley, New York.

Tikhonovich, I.A., and N.A. Provorov. 2007. Cooperation of plants and microorganisms: Getting closer to the genetic construction of sustainable agro-systems. Biotechnol. J. 2:833-848.

Toro, M., R. Azcón, and J. M. Barea, .1998. The use of isotopic dilution techniques to evaluate the interactive effects of Rhizobium genotype, mycorrhizal fungi, phosphate-solubilizing rhizobacteria and rock phosphate on nitrogen and phosphorus acquisition by *Medicago sativa*. New Phytol. 138:265–273

Toro, M., R. Azcón, and J.M. Barea. 1997. Improvement of arbuscular mycorrhiza development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (^{32}P) and nutrient cycling. Appl. Environ. Microbiol. 63:4408–4412.

Torres-Barraga´n, A., E. Zavale-Tamejia, C. Gonzalez-Chavez, and R. Ferrera-Cerrato. 1996. The use of arbuscular mycorrhizae to control onion white rot (*Sclerotium cepivorum*) under field conditions. Mycorrhizae 6:253–257.

Tuinen, D. van, E. Jacquot, B. Zhao, A. Gollotte, and V. Gianinazzi-Pearson. 1998. Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. Mol. Ecol. 7:879–887

Unkovich, M. J., and J. S. Pate. 2000. An appraisal of recent field measurements of symbiotic N_2 fixation by annual legumes. Field Crops Res. 65:211-228.

Valdenegro, M., J. M. Barea, and R. Azcón. 2001. Influence of arbuscular mycorrhizal fungi, Rhizobium meliloti strains and PGPR inoculation on the growth of *Medicago arborea* used as model legume for re-vegetation and biological reactivation in a semi-arid mediterranean area. Plant Growth Regul. 34:233–240

Van der Heijden, M.G.A., A. Wiemken, and I.R. Sanders. 2003. Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plants. New Phytol. 157:569–578.

van der Heijden, M.G.A., R. Streitwolf-Engel, R. Riedl, S. Siegrist, A. Neudecker, K. Ineichen, T. Bolder, A. Wiemen, and I.R. Sanders. 2006. The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. New Phytol. 172:739-752.

Vazquez, M.M., S. Cesar, R. Azcòn, and J.M. Barea. 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum, Pseudomonas, Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Appl. Soil Ecol. 15:261-272.

Venkateswarlu, B., M. Pirat, N. Kishore, and A. Rasul. 2008. Mycorrhizal inoculation in neem (*Azadirachta indica)* enhances azadirachtin content in seed kernels. World J. Microb. Biot. 24:1243–1247.

Vesjsadova, H., D. Siblikova, M. Gryndler, T. Simon, and I. Miksik. 1993. Influence of inoculation with *Bradyrhizobium japonicum* and *Glomus claroideum* on seed yield of soybean under greenhouse and field conditions. J. Plant Nutr. 16:619–629.

Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 255:571-586.

Vierheilig, H., A.P. Coughlan, U. Wyss, and Y. Piché. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ. Microbiol. 64: 5004-5007.

Vierheilig, H., and Y. Piché. 2002. Signalling in arbuscular mycorrhiza: facts and hypothesis. In Flavonoids in cell function. Eds. Buslig, B., and J. Manthley. Kluwer Academic/Plenum Publ.,New York. p. 23–39.

Vivas A., A. Marulanda, J.M. Ruiz-Lozano, J.M. Barea and R. Azcon. 2003. Influence of a *Bacillus* sp on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG-induced drought stress. Mycorrhiza, 13:249-256.

Vogelsang, K.M., H.L. Reynolds, J.D. Bever. 2006. Mycorrhizal fungal identity and richness determine the diversity and productivity of a tall grass prairie system. New Phytologist. 172:554-562.

Von Alten, H., A. Lindermann, F. Schonbeck. 1993. Stimulation of vesicular–arbuscular mycorrhiza by fungicides or rhizosphere bacteria. Mycorrhiza 2:167–173

Von Alten, H., B. Blal, J.C. Dodd, F. Feldmann, and M.Vosatka. 2002. Quality Control of arbuscular mycorrhizal inoculum in Europe. In: Mycorrhizal Technology in Agriculture - From Genes to Bioproducts. Birkhäuser Verlag: Basel.

Vosatka, M. 1995. Influence of inoculation with arbuscular mycorrhizal fungi on the growth and mycorrhizal infection of transplanted onion. Agric. Ecosyst. Environ. 53:151–159.

Wang, B., and Y.L. Qiu. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363

Weissenhorn, I., C. Leyval, and J. Berthelin. 1993. Cd-tolerant arbuscular mycorrhizal fungi from heavy metal polluted soils. Plant Soil. 157:247–256

Weissenhorn, I., M. Mench, and C. Leyval. 1995. Bioavailability of heavy metals and abundance of arbuscular mycorrhiza in a sewage sludge amended sandy soil. Soil Biol. Biochem. 27:287–296

Withers, P.J.A., A.C. Edwards, and R.H. Foy. 2001. Phosphorus cycling in UK agriculture and implications for phosphorus loss from soil. Soil Use Manage. 17:139–149.

Xavier, L.J. C. and J.J. Germida. 1997. Growth response of lentil and wheat to *Glomus clarum* NT4 over a range of P levels in a Saskatchewan soil containing indigenous AM fungi. Mycorrhiza. 7:3-8.

Xavier, L.J.C., and J.J. Germida. 2002. Response of lentil to co-inoculation with arbuscular mycorrhizal fungi and rhizobia varying in efficacy, Soil. Biol. Biochem. 34:181–188.

Xavier, L.J.C., and J.J. Germida. 2003. Selective interactions between arbuscular mycorrhizal fungi and *Rhizobium leguminosarum* bv. *viceae* enhance pea yield and nutrition, Biol. Fertil. Soils. 37:261–267

Xie, Z.P., C. Staehelin, H. Vierheilig, A. Wiemken, S. Jabbouri, W.J. Broughton, R. Vogeli-Lange, and T. Boller. 1995. Rhizobial nodulation factors stimulate mycorrhizal colonization of nodulating and nonnodulating soybeans. Plant Physiology. 108:1519-1525.

Yuan, Z.L., C.C. Dai, and L.Q. Chen. 2007. Regulation and accumulation of secondary metabolites in plant-fungus symbiotic system. Afr. J. Biotech. 6:1266–1271.

Zhang, L., and Z.H. Xu. 2008. Assessing bacterial diversity in soil: a brief review. J Soils Sediments 8:379–388

Zhu, Y., and R.M. Miller. 2003. Carbon cycling by arbuscular mycorrhizal fungi in soil–plant systems. Trends Plant Sci. 8:407-409.

8. APPENDICES

8.1 Appendix A: Effects of AMF, *Rhizobium* and phosphorus treatments and their interactions on AMF colonization, nodulation, biomass, yield and seed properties of field pea in 2012 and 2013.

Table. A.1. Combined site analysis for effect of AMF, *Rhizobium* and phosphorus treatments and their interactions on AMF colonization and nodulation of field pea in 2012 and 2013.

AMF†	*Rhizobium*‡	Phosphorus§ (kg ha^{-1})	AMF colonization (%)	Nodulation (No. per plant)
2012 Field Season				
0X	1X	0	50a¶	49d
		16.8	56a	61c
1X	1X	0	63a	66c
		16.8	65a	141a
2X	1X	0	55a	63c
		16.8	62a	80b
2013 Field Season				
0X	0X	0	62a	55c
		16.8	65a	66b
0X	1X	0	59a	55c
		16.8	65a	78b
1X	0X	0	67a	60b
		16.8	63a	75b
1X	1X	0	73a	67b
		16.8	71a	105a
2X	0X	0	69a	42c
		16.8	61a	57bc
2X	1X	0	70a	45c
		16.8	67a	73b

† Three levels of AMF (arbuscular mycorrhizal fungi) treatment, 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively

‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, 0 kg ha^{-1} and recommended rate are designated as 0X and 1X

§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate

¶ Means followed by the same lower case letter in the same column are not significantly different (p<0.05). The multi-treatment comparisons were made using the Tukey's HSD method.

Table A.2. Combined site analysis for effect of AMF, Rhizobium and phosphorus treatments and their interactions on AMF colonization and nodulation of lentil in 2012 and 2013.

AMF†	*Rhizobium*‡	Phosphorus§ (kg ha^{-1})	AMF colonization (%)	Nodulation (No. per plant)
2012 Field Season				
0X	1X	0	42a¶	72c
		16.8	50a	89b
1X	1X	0	56a	97b
		16.8	53a	123a
2X	1X	0	55a	83bc
		16.8	48a	92b
2013 Field Season				
0X	0X	0	58a	50c
		16.8	61a	56c
0X	1X	0	55a	40d
		16.8	59a	61b
1X	0X	0	64a	63b
		16.8	57a	72b
1X	1X	0	68a	62bc
		16.8	65a	97a
2X	0X	0	68a	40d
		16.8	59a	46cd
2X	1X	0	67a	54c
		16.8	60a	57c

† Three levels of AMF (arbuscular mycorrhizal fungi) treatment, 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively

‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, 0 kg ha^{-1} and recommended rate are designated as 0X and 1X

§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate

¶ Means followed by the same lower case letter in the same column are not significantly different (p<0.05). The multi-treatment comparisons were made using the Tukey's HSD method.

Table A.3. Analyses of the impact of AMF, P treatments in 2012 on post-harvest MPN of AMF of field pea and lentil.

Treatments	Field Pea				Lentil	
	Kelvington		Stewart Valley		Stewart Valley	
	F[†]	*p*[†]	F	*p*	F	*P*
2012 Field Season						
AMF[‡]	13.5	**0.021**	7.3	**0.034**	6.2	**0.038**
P[§]	0.9	0.883	0.3	0.173	0.3	0.833
AMF × P[¶]	1.3	0.926	0.6	0.903	1.2	0.705
Contrast[#]						
0X AMF vs 1X, 2X AMF	1.1	0.801	0.3	0.672	0.6	0.089
0X AMF vs 1X AMF	0.9	0.652	0.8	0.534	0.9	0.331
0X AMF vs 2X AMF	0.6	0.837	0.2	0.793	2.1	0.215

† F and *p* values for treatment effects and interaction terms and orthogonal comparison derived from an ANOVA ($p<0.05$).

‡ Three levels of AMF treatment are 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.

§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.

¶ AMF and P interaction.

Orthogonal class contrast = Class comparison of AMF application rates (7.5 kg ha^{-1} and 15 kg ha^{-1}, or average of 7.5 kg ha^{-1} and 15 kg ha^{-1}) as a class vs. none applied.

Table A.4. Analyses of the impact of AMF, *Rhizobium* and phosphorus treatments in 2013 on post-harvest MPN of AMF of field pea and lentil.

Treatments	Field pea						Lentil	
	Stewart Valley		Pampbrun		Outlook		Stewart Valley	
	F†	*p*†	F	*p*	F	*p*	F	*p*
2013 Field Season								
AMF‡	7.9	**0.028**	9.1	**0.041**	5.8	**0.044**	10.4	**0.037**
P§	1.2	0.758	0.9	0.777	0.8	0.993	1.6	0.861
Rhizobium¶	2.5	0.071	3.1	0.670	1.9	0.081	3.6	0.104
AMF × P#	0.9	0.592	0.8	0.988	1.9	0.741	1.4	0.726
AMF × *Rhizobium*††	4.7	0.096	3.3	0.122	2.8	0.582	2.1	0.148
P × *Rhizobium*‡‡	3.0	0.209	2.1	0.361	4.3	0.089	1.8	0.737
AMF × P × *Rhizobium*§§	2.5	0.582	1.6	0.673	0.5	0.326	0.8	0.803
Contrast¶¶								
0X AMF vs 1X, 2X AMF	0.3	0.437	1.8	0.566	1.3	0.847	0.9	0.451
0X AMF vs 1X AMF	0.9	0.783	1.4	0.782	1.3	0.652	2.1	0.777
0X AMF vs 2X AMF	0.6	0.931	0.6	0.677	1.5	0.378	1.2	0.839

† F and *p* values for treatment effects and interaction terms and orthogonal comparison derived from an ANOVA ($p<0.05$).

‡ Three levels of AMF treatment are 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.

§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate...

¶ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, control and recommended rate were applied.

AMF and P interactions

†† AMF and *Rhizobium* interactions.

‡‡ P and *Rhizobium* interactions.

§§ AMF, P and *Rhizobium* interactions.

¶¶ Orthogonal class contrast = Class comparison of AMF application rates (7.5 kg ha^{-1} and 15 kg ha^{-1}, or average of 7.5 kg ha^{-1} and 15 kg ha^{-1}) as a class vs. none applied.

Table A.5. Effect of AMF, *Rhizobium* and phosphorus treatments and their interactions on MPN of field pea and lentil at different sites in 2012.

			Field Pea		Lentil
AMF†	***Rhizobium***‡	**Phosphorus**§ **(kg ha^{-1})**	**MPN (spores 100 g^{-1} soil)**		
			Kelvington	**Stewart Valley**	**Stewart Valley**
0X	1X	0	123b¶	94b	99b
		16.8	127b	98b	103b
1X	1X	0	166a	130b	135a
		16.8	129b	111ab	117b
2X	1X	0	134b	104b	108b
		16.8	141b	98b	103b

† Three levels of AMF (arbuscular mycorrhizal fungi) treatment, 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.

‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, 0 kg ha^{-1} and recommended rate, designated as 0X and 1X

§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.

¶ Means followed by the same lower case letter in the same column are not significantly different (p<0.05). Multi-treatment comparisons made using the Tukey's HSD method.

Table A.6. Effect of AMF, *Rhizobium* and phosphorus treatments and their interactions on MPN of field pea and lentil at different sites in 2013.

			Field pea			Lentil
AMF[†]	***Rhizobium***[‡]	**Phosphorus**[§] **(kg ha^{-1})**	**MPN (spores 100 g^{-1} soil)**			
			Stewart Valley	**Outlook**	**Pampbrun**	**Stewart Valley**
0X	0X	0	93b	77b	87b	98b
		16.8	106b	78b	93b	92b
0X	1X	0	89b	85b	94b	101b
		16.8	93b	83b	88b	97b
1X	0X	0	143a	123a	125a	151a
		16.8	137a	116a	113ab	149a
1X	1X	0	126ab	94b	106b	133ab
		16.8	118b	106ab	110ab	118b
2X	0X	0	123ab	102ab	99b	106b
		16.8	111b	91b	101b	124b
2X	1X	0	119b	87b	103b	112b
		16.8	110b	83b	85b	103b

† Three levels of AMF (arbuscular mycorrhizal fungi) treatment; 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.
‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, 0 kg ha^{-1} and recommended rate are designated as 0X and 1X.
§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.
¶ Means followed by the same letter in the same column are not significantly different (p<0.05). The multi-treatment comparisons were made using the Tukey's HSD method.

119

Table A.7. Effect of AMF, *Rhizobium* and phosphorus treatments and their interactions on biomass and plant nutrient uptake of pea (combined between sites) in 2012 and 2013.

AMF[†]	*Rhizobium*[‡]	Phosphorus[§] (kg ha^{-1})	Mid-season Biomass			Final Biomass		
			Biomass (kg ha^{-1})	N (kg ha^{-1})	P (kg ha^{-1})	Biomass[#] (kg ha^{-1})	N[#] (kg ha^{-1})	P[#] (kg ha^{-1})
2012 Field Season-Pea								
0X	1X	0	2679.4a[¶]	69.3b	6.7c	-	-	-
		16.8	2623.7a	72.8ab	7.9b	-	-	-
1X	1X	0	2756.9a	68.9b	7.5b	-	-	-
		16.8	2791.4a	83.5a	8.5a	-	-	-
2X	1X	0	2722.8a	62.5b	7.8b	-	-	-
		16.8	2704.4a	66.8b	7.9b	-	-	-
2013 Field Season-Pea								
0X	0X	0	4002.1c	121.1c	12.5c	1126.5a	66.4a	9.8a
		16.8	4436.7b	130.3b	15.7c	1265.4a	65.3a	7.1a
0X	1X	0	4302.4b	138.9b	16.1c	1267.5a	57.3a	8.2a
		16.8	4759.1a	163.3a	21.3bc	1374.0a	61.2a	6.1a
1X	0X	0	4206.7bc	142.2bc	24.2b	1156.9a	59.3a	7.8a
		16.8	4202.3bc	145.6bc	30.9a	1253.0a	61.5a	9.3a
1X	1X	0	4409.4b	153.2b	28.3a	1333.9a	63.8a	8.9a
		16.8	4902.1a	169.7a	29.2a	1403.7a	55.9a	9.3a
2X	0X	0	4056.5c	123.2c	20.1bc	1198.3a	58.2a	6.2a
		16.8	4308.2b	136.4b	24.1b	1237.2a	63.2a	7.4a
2X	1X	0	4046.1c	121.5c	11.3c	1103.4a	49.1a	9.1a
		16.8	4146.7c	124.8c	13.7c	1047.5a	57.2a	7.2a

† Three levels of AMF (arbuscular mycorrhizal fungi) treatment, 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.
‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, 0 kg ha^{-1} and recommended rate, designated as 0X and 1X
§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.
¶ Means followed by the same lower case letter in the same column are not significantly different (p<0.05). Multi-treatment comparisons made using the Tukey's HSD method.
# Final biomass was not taken for field pea in 2012.

Table A.8. Effect of AMF, *Rhizobium* and phosphorus treatments and their interactions on biomass and plant nutrient uptake of lentil (combined between sites) in 2012 and 2013.

AMF†	*Rhizobium*‡	Phosphorus§ (kg ha^{-1})	Mid-season Biomass			Final Biomass		
			Biomass¶ (kg ha^{-1})	N (kg ha^{-1})	P (kg ha^{-1})	Biomass (kg ha^{-1})	N (kg ha^{-1})	P (kg ha^{-1})
2012 Field Season-Lentil								
0X	1X	0	1089.9a¶	41.5c	4.8c	683.2a	11.6a	2.1a
		16.8	1113.5a	47.9bc	5.5b	691.7a	12.9a	2.3a
1X	1X	0	1106.1a	54.1b	5.9b	693.5a	11.0a	1.9a
		16.8	1291.9a	63.8a	7.5a	704.8a	13.1a	2.1a
2X	1X	0	1092.4a	52.5b	4.9c	682.4a	12.2a	1.2a
		16.8	1004.6a	46.8bc	5.9b	684.1a	10.8a	1.9a
2013 Field Season-Lentil								
0X	0X	0	3103.2c	93.7c	8.2c	1090.6a	51.2a	5.2a
		16.8	3513.1b	120.1b	9.5c	1031.5a	56.1a	5.8a
0X	1X	0	3512.0b	111.7b	9.0c	1101.2a	53.4a	5.1a
		16.8	3800.9a	135.7a	15.2b	1104.5a	57.2a	5.7a
1X	0X	0	3290.7bc	102.1bc	14.7b	1066.8a	50.2a	6.6a
		16.8	3859.7.3a	118.3b	15.4b	1107.0a	55.8a	6.2a
1X	1X	0	3421.0b	121.2b	19.8ab	1167.9a	51.8a	7.1a
		16.8	3993.7a	140.2a	26.9a	1003.1a	57.8a	6.9a
2X	0X	0	3141.9c	103.2bc	13.8bc	1098.0a	52.5a	5.2a
		16.8	3211.5bc	121.4b	15.1b	1103.9a	52.1a	6.2a
2X	1X	0	3107.9c	106.1bc	12.7bc	1001.2a	55.0a	7.1a
		16.8	3209.0bc	93.1c	7.0c	1197.9a	50.6a	5.6a

† Three levels of AMF (arbuscular mycorrhizal fungi) treatment; 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.
‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, 0 kg ha^{-1} and recommended rate are designated as 0X and 1X.
§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.
¶ Means followed by the same letter in the same column are not significantly different (p<0.05). The multi-treatment comparisons were made using the Tukey's HSD method.

Table A.9. Effect of AMF, *Rhizobium* and phosphorus treatments and their interactions on seed yield (small plot combine) and seed quality parameters of pea at Stewart Valley in 2012 and combined between sites in 2013.

2012 Field Season-Pea					
AMF[†]	***Rhizobium***[‡]	**Phosphorus**[§] **(kg ha^{-1})**	**Seed quality parameters**		
			Yield (kg ha^{-1})	**N (kg ha^{-1})**	**P (kg ha^{-1})**
0X	1X	0	1044.1a[¶]	41.08bc	4.42c
		16.8	1148.9a	47.76b	5.98bc
1X	1X	0	1133.1a	47.00b	6.33b
		16.8	1358.9a	63.07a	9.67a
2X	1X	0	1002.4a	39.16c	4.28c
		16.8	1105.8a	42.08bc	6.23b
2013 Field Season-Pea					
0X	0X	0	3177.3a	79.64d	8.34c
		16.8	3313.9a	79.93d	10.26abc
0X	1X	0	3459.1a	100.98a	8.53c
		16.8	3811.6a	101.29a	12.62a
1X	0X	0	3356.2a	84.77bc	8.48c
		16.8	3301.5a	93.12bc	9.81bc
1X	1X	0	3577.1a	95.64bc	9.56bc
		16.8	3968.8a	107.99a	13.22a
2X	0X	0	3266.1a	87.05bcd	8.99c
		16.8	3351.8a	93.65bc	9.25bc
2X	1X	0	3379.6a	86.25bcd	9.91bc
		16.8	3288.3a	87.76bcd	9.56bc

† Three levels of AMF (arbuscular mycorrhizal fungi) treatment; 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.
‡ In 2012, *Rhizobium* was used in all treatments. In 2013 two levels of *Rhizobium*, 0 kg ha^{-1} and recommended rate are designated as 0X and 1X.
§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.
¶ Means followed by the same lower case letter in the same column are not significantly different (p<0.05). The multi-treatment comparisons were made using the Tukey's HSD method.

Table A.10. Effect of AMF, *Rhizobium* and phosphorus treatments and their interactions on seed yield (small plot combine) and seed quality parameters of lentil at Stewart Valley in 2012 and 2013.

2012 Field Season-Lentil					
AMF[†]	***Rhizobium***[‡]	**Phosphorus**[§] **(kg ha^{-1})**	**Seed quality parameters**		
			Yield (kg ha^{-1})	**N (kg ha^{-1})**	**P (kg ha^{-1})**
0X	1X	0	892.5a[¶]	26.71c	3.45c
		16.8	934.1a	31.04b	4.82bc
1X	1X	0	913.7a	39.02b	5.58b
		16.8	993.1a	50.71a	7.32a
2X	1X	0	817.8a	24.55c	3.17c
		16.8	921.5a	26.67c	3.24c
2013 Field Season-Lentil					
0X	0X	0	1821.7a	47.58bc	5.36bc
		16.8	2041.1a	53.71b	6.07b
0X	1X	0	1901.3a	62.33a	6.87b
		16.8	2302.6a	66.39a	7.02a
1X	0X	0	1988.3a	54.94b	5.04bc
		16.8	2100.0a	61.17a	5.41b
1X	1X	0	2082.3a	53.04b	5.46b
		16.8	2499.2a	70.73a	8.67a
2X	0X	0	1862.1a	45.49c	4.39c
		16.8	1987.3a	53.05b	5.22b
2X	1X	0	1752.9a	36.08c	3.21c
		16.8	1821.3a	40.98c	4.93c

† Three levels of AMF(arbuscular mycorrhizal fungi) treatment; 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.
‡ In 2012, *Rhizobium* was used in all treatments. In 2013 two levels of *Rhizobium*, 0 kg ha^{-1} and recommended rate are designated as 0X and 1X.
§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.
¶ Means followed by the same lower case letter in the same column are not significantly different (p<0.05). The multi-treatment comparisons were made using the Tukey's HSD method.

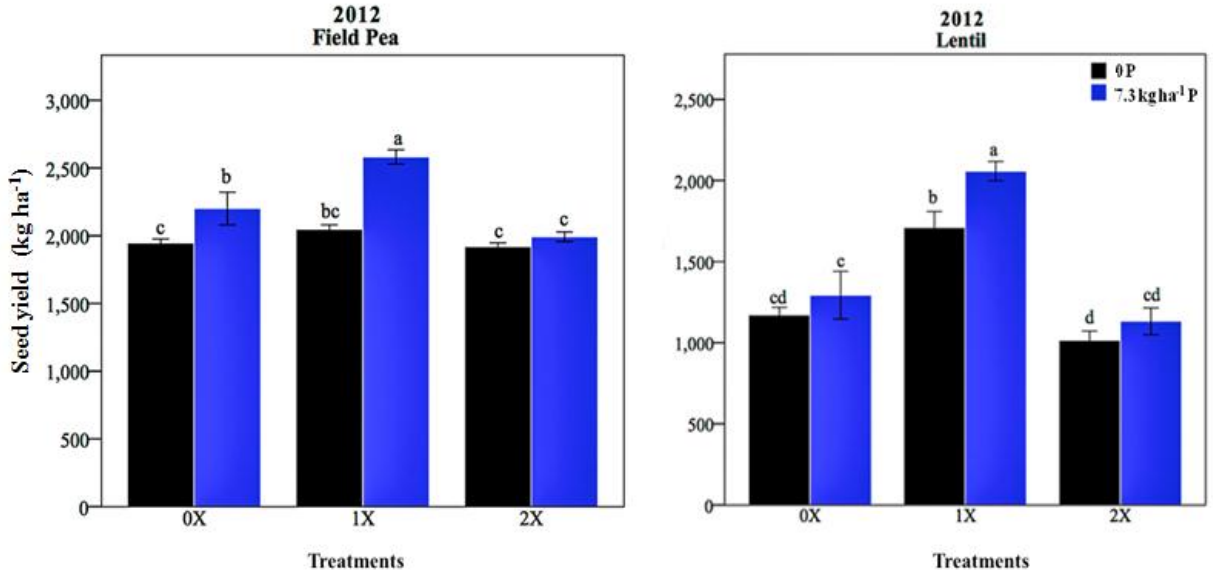


Fig A.1. Mean seed yield (hand harvest) in kg ha^{-1} in field pea at Kelvington and lentil at Stewart Valley in 2012. Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 were applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

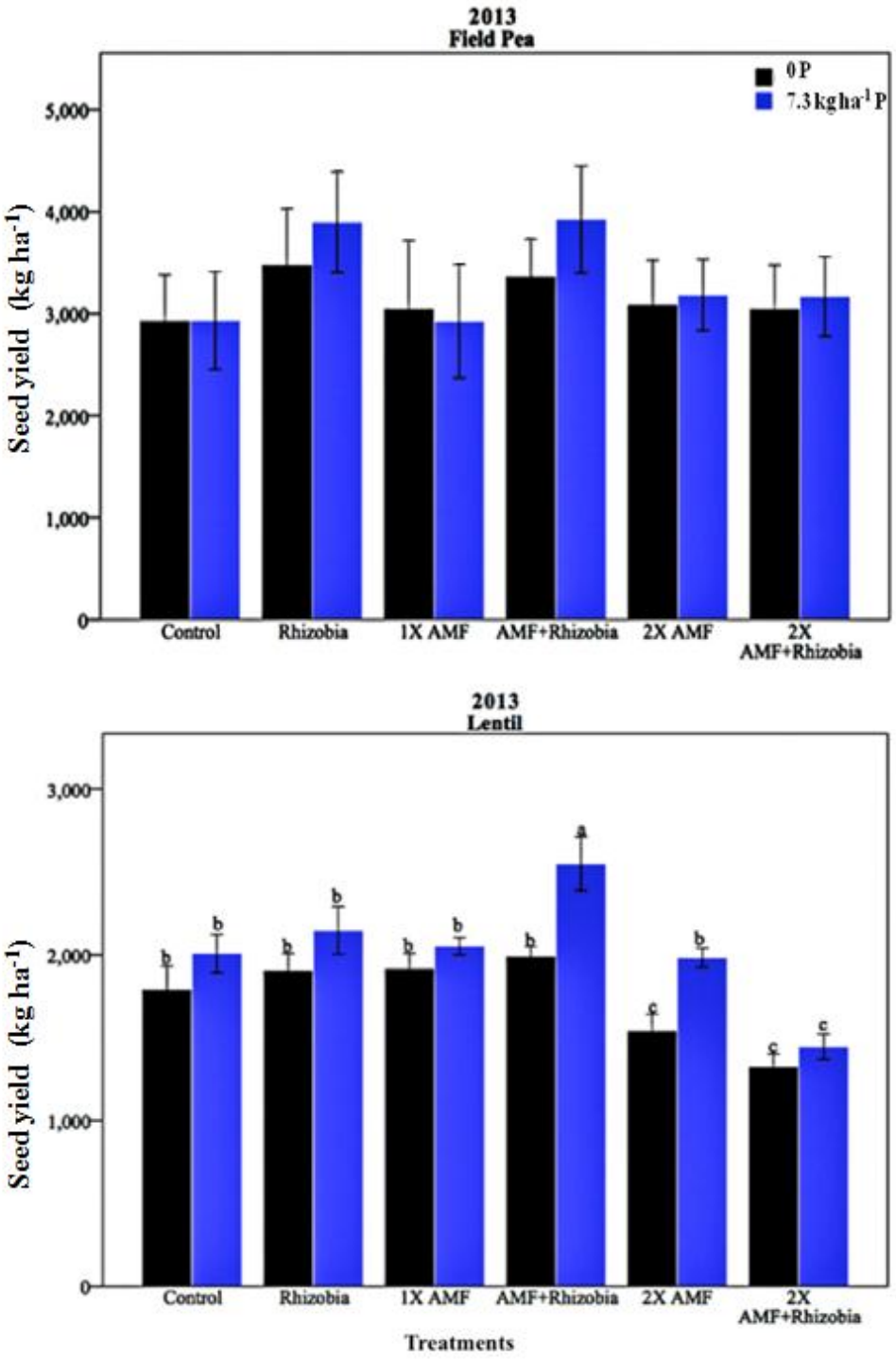


Fig A.2. Mean seed yield (hand harvest) in kg ha^{-1} in field pea and lentil in 2013 (averaged across all sites for field pea and at Stewart Valley for lentil). Three levels of arbuscular mycorrhizal fungi (AMF), 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 was applied as monoammonium phosphate. Error bars represent ± standard errors of the mean. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

Table A.11. Combined site analyses of the impact of AMF, P treatments in 2012, and AMF *Rhizobium* and phosphorus treatments in 2013 on seed yield (hand harvest) of field pea and lentil.

Treatments	Field Pea		Lentil	
	Seed Yield		Seed Yield	
	F[†]	*p*[†]	F	*p*
2012 Field Season				
AMF[‡]	0.3	0.703	1.5	0.436
P[§]	6.1	**0.038**	6.2	**0.039**
AMF × P[¶]	7.8	**0.043**	11.8	**0.030**
Contrast[#]				
0X AMF vs 1X, 2X AMF	0.6	0.586	7.6	**0.049**
0X AMF vs 1X AMF	4.1	**0.046**	18.9	**0.031**
0X AMF vs 2X AMF	0.2	0.872	1.2	0.122
2013 Field Season				
AMF	0.6	0.893	0.6	0.367
P	1.3	0.249	5.1	**0.049**
Rhizobium[††]	0.4	0.730	0.2	0.877
AMF × P	1.1	0.339	4.1	**0.039**
AMF × *Rhizobium*[‡‡]	0.8	0.522	0.3	0.773
P × *Rhizobium*[§§]	0.4	0.775	0.1	0.237
AMF × P × *Rhizobium*[¶¶]	1.6	0.216	9.6	**0.046**
Contrast				
0X AMF vs 1X, 2X AMF	0.2	0.758	0.2	0.878
0X AMF vs 1X AMF	0.1	0.839	0.5	0.549
0X AMF vs 2X AMF	1.4	0.194	1.4	0.241

† F and *p* values for treatment effects and interaction terms and orthogonal comparison derived from an ANOVA ($p<0.05$).

‡ Three levels of AMF treatment are 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively.

§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.

¶ AMF and P interactions.

Orthogonal class contrast = Class comparison of AMF application rates (7.5 kg ha^{-1} and 15 kg ha^{-1}, or average of 7.5 kg ha^{-1} and 15 kg ha^{-1}) as a class vs. none applied.

†† In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, control and recommended rate were applied.

‡‡ AMF and *Rhizobium* interactions.

§§ P and *Rhizobium* interactions.

¶¶ AMF, P and *Rhizobium* interactions.

Table A.12. Combined site analyses of the impact of AMF, P treatments in 2012, and AMF *Rhizobium* and phosphorus treatments in 2013 on seed yield (hand harvest) of field pea and lentil.

AMF†	*Rhizobium*‡	Phosphorus§ (kg ha^{-1})	Field pea Yield (kg ha^{-1})	Lentil Yield (kg ha^{-1})
2012 Field Season				
0X	1X	0	1929.8c¶	1172.5cd¶
		16.8	2153.4bc	1373.9c
1X	1X	0	2004.1bc	1666.4b
		16.8	2603.5a	2128.8a
2X	1X	0	1901.6c	1032.1d
		16.8	1940.2c	1107.5d
2013 Field Season				
0X	0X	0	2839.6a	1709.2b
		16.8	2979.3a	1991.3b
0X	1X	0	3642.8a	1868.2b
		16.8	3878.5a	2288.1b
1X	0X	0	2937.0a	1837.6b
		16.8	3178.9a	2052.9b
1X	1X	0	3352.0a	1952.5b
		16.8	3904.2a	2634.7a
2X	0X	0	2972.9a	1552.9c
		16.8	3293.5a	1929.5b
2X	1X	0	2956.1a	1269.1c
		16.8	3164.0a	1444.7c

† Three levels of AMF (arbuscular mycorrhizal fungi) treatment, 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively

‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, 0 kg ha^{-1} and recommended rate are designated as 0X and 1X

§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate

¶ Means followed by the same lower case letter in the same column are not significantly different (p<0.05). The multi-treatment comparisons were made using the Tukey's HSD method.

Table A.13. Combined site analysis for effect of AMF, *Rhizobium* and phosphorus treatments and their interactions on % N directly fixed from atmosphere (%Ndfa) and biologically fixed N of field pea in 2012 and 2013.

2012 Field Season				
AMF[†]	***Rhizobium***[‡]	**Phosphorus**[§] **(kg ha^{-1})**	**% N dfa**	**Total N fixed (kg ha^{-1})**
0X	1X	0	44.5b[¶]	31.38c
		16.8	56.6b	36.04bc
1X	1X	0	50.3b	42.56b
		16.8	69.2a	58.72a
2X	1X	0	52.1b	30.25c
		16.8	43.1b	29.78c
2013 Field Season				
0X	0X	0	54.3c	73.03c
		16.8	54.1c	70.43c
0X	1X	0	54.8c	75.28c
		16.8	72.1a	105.57ab
1X	0X	0	56.2c	87.64b
		16.8	79.1a	115.16a
1X	1X	0	62.1bc	95.12b
		16.8	77.3a	131.81a
2X	0X	0	53.1c	65.42c
		16.8	61.2bc	83.48bc
2X	1X	0	55.5c	67.25c
		16.8	56.3c	70.24c

† Three levels of AMF (arbuscular mycorrhizal fungi) treatment are 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively

‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, 0 kg ha^{-1} and recommended rate are designated as 0X and 1X

§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate

¶ Means followed by the same lower case letter in the same column are not significantly different (p<0.05). Multi-treatment comparisons were made using the Tukey's HSD method

Table A.14. Combined site analysis for effect of AMF, Rhizobium and phosphorus treatments and their interactions on % N directly fixed from atmosphere (%Ndfa) and biologically fixed N in lentil in 2012 and 2013.

2012 Field Season				
AMF[†]	***Rhizobium***[‡]	**Phosphorus**[§] **(kg ha^{-1})**	**% N dfa**	**Total N fixed (kg ha^{-1})**
0X	1X	0	39.5bc	21.95c
		16.8	51.6b	29.16b
1X	1X	0	39.2bc	21.72c
		16.8	62.9a	40.02a
2X	1X	0	30.8c	19.17c
		16.8	40.7b	20.47c
2013 Field Season				
0X	0X	0	48.3c	42.71c
		16.8	51.6bc	59.16b
0X	1X	0	51.2bc	51.04bc
		16.8	63.7ab	78.69b
1X	0X	0	57.4b	46.34c
		16.8	67.9a	78.77b
1X	1X	0	58.3b	70.96b
		16.8	72.3a	97.86a
2X	0X	0	52.7bc	54.34bc
		16.8	58.6b	71.14b
2X	1X	0	50.8c	53.88bc
		16.8	52.3bc	55.03bc

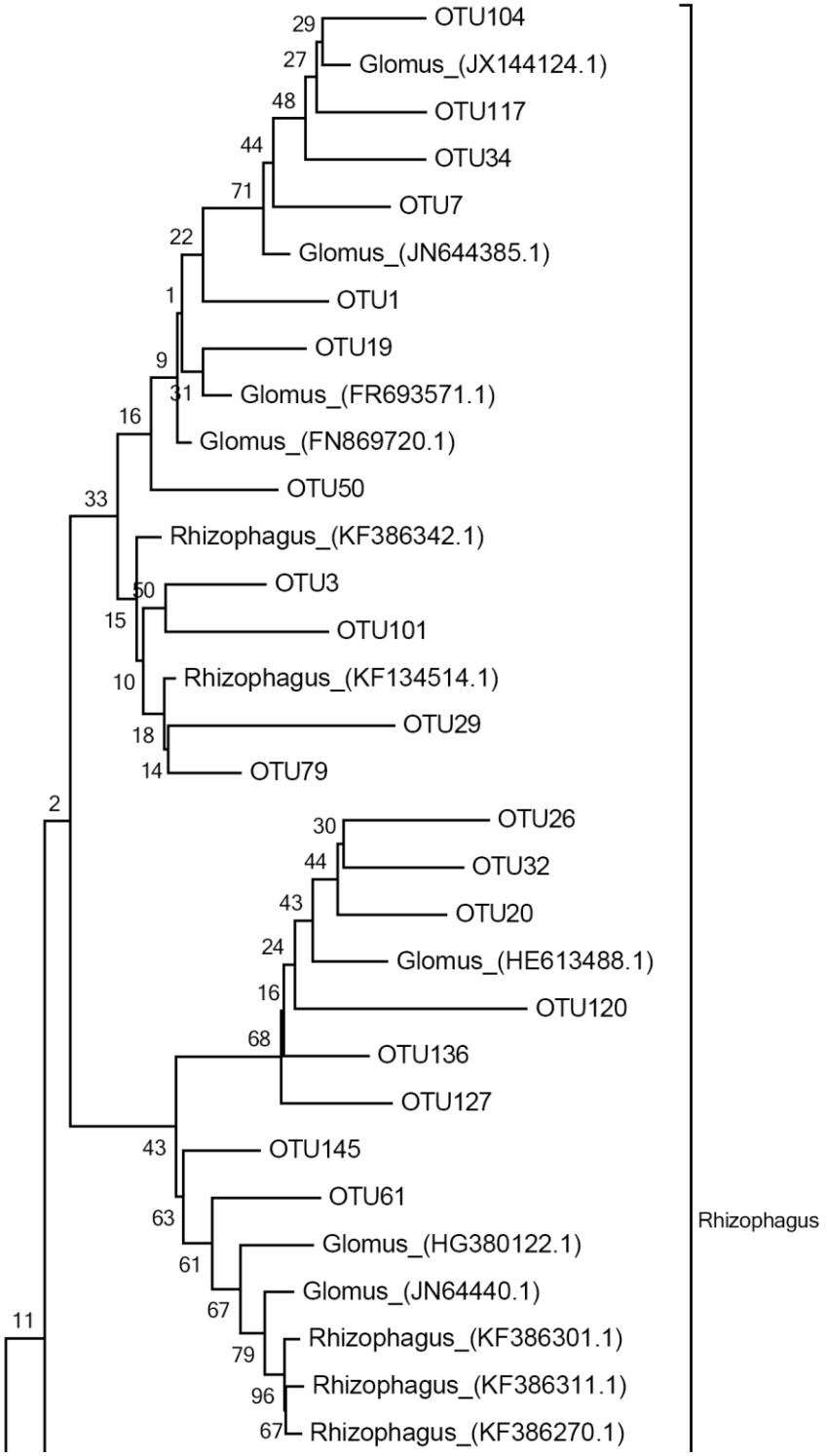
† Three levels of AMF (arbuscular mycorrhizal fungi) treatment are 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively

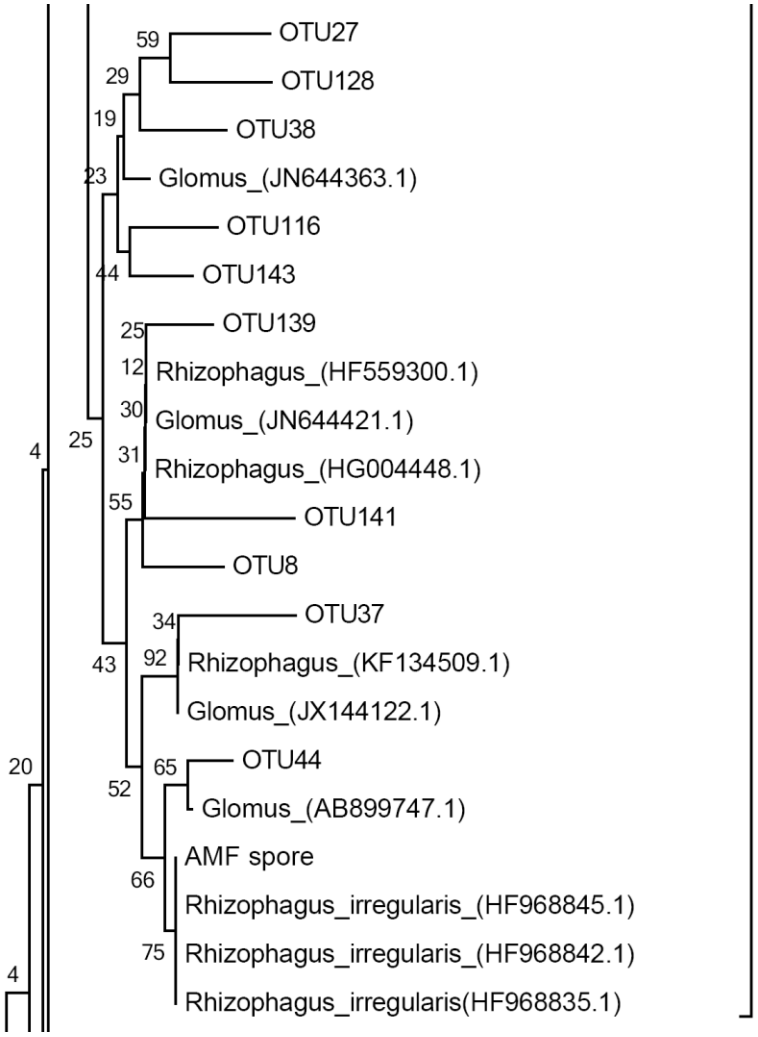
‡ In 2012, *Rhizobium* was used across all treatments. In 2013 two levels of *Rhizobium*, 0 kg ha^{-1} and recommended rate are designated as 0X and 1X

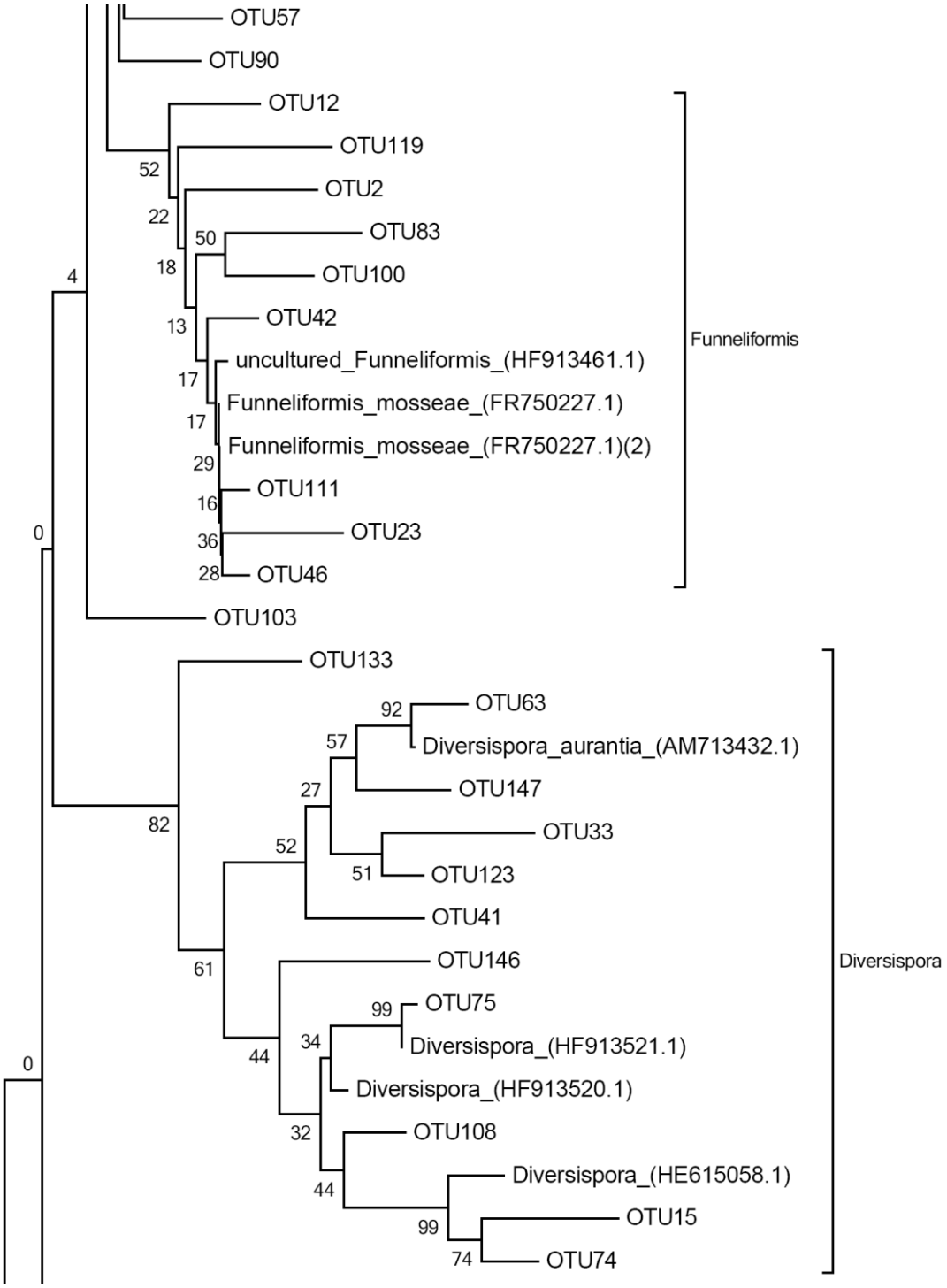
§ Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate

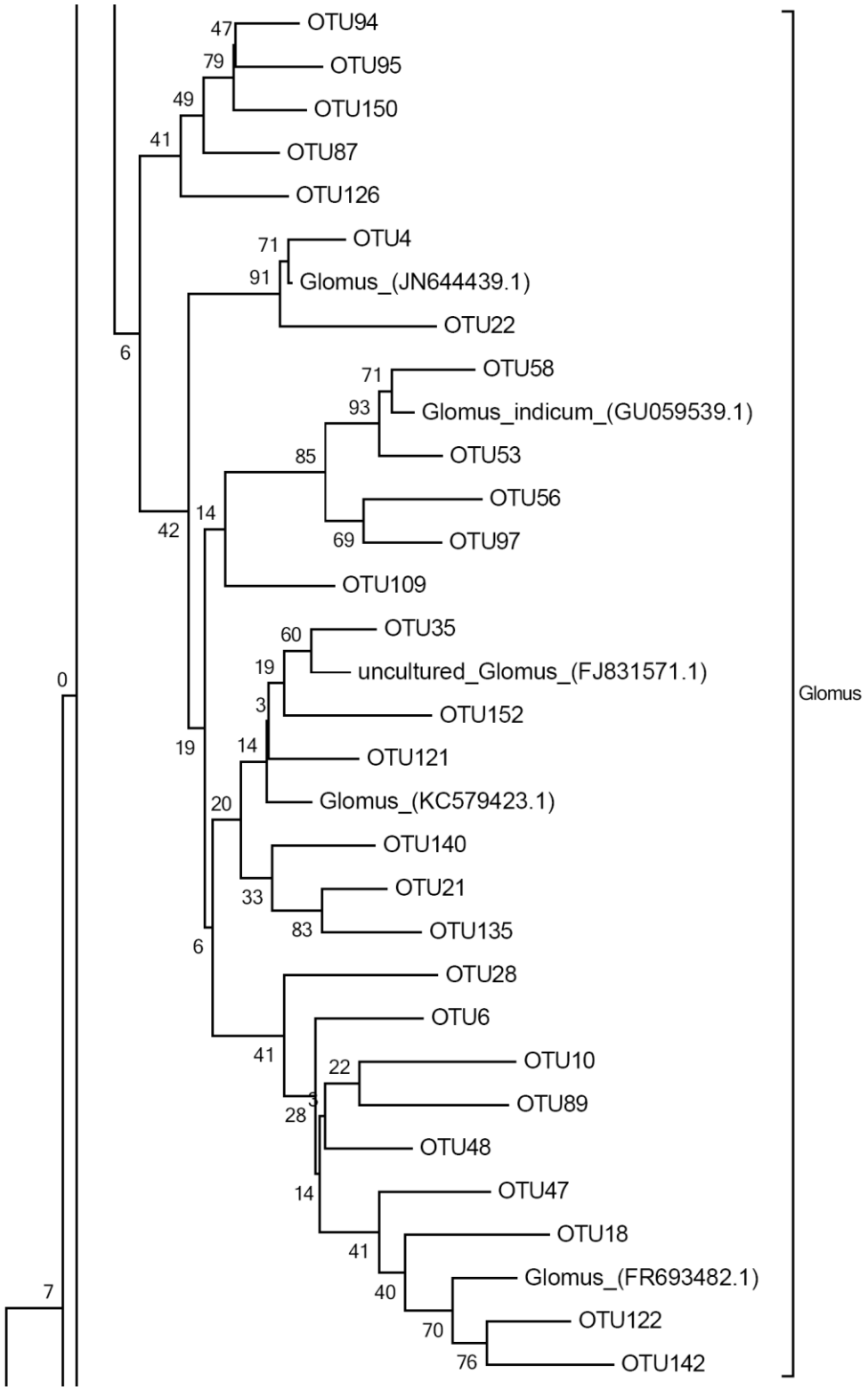
¶ Means followed by the same lower case letter in the same column are not significantly different (p<0.05). Multi-treatment comparisons were made using the Tukey's HSD method.

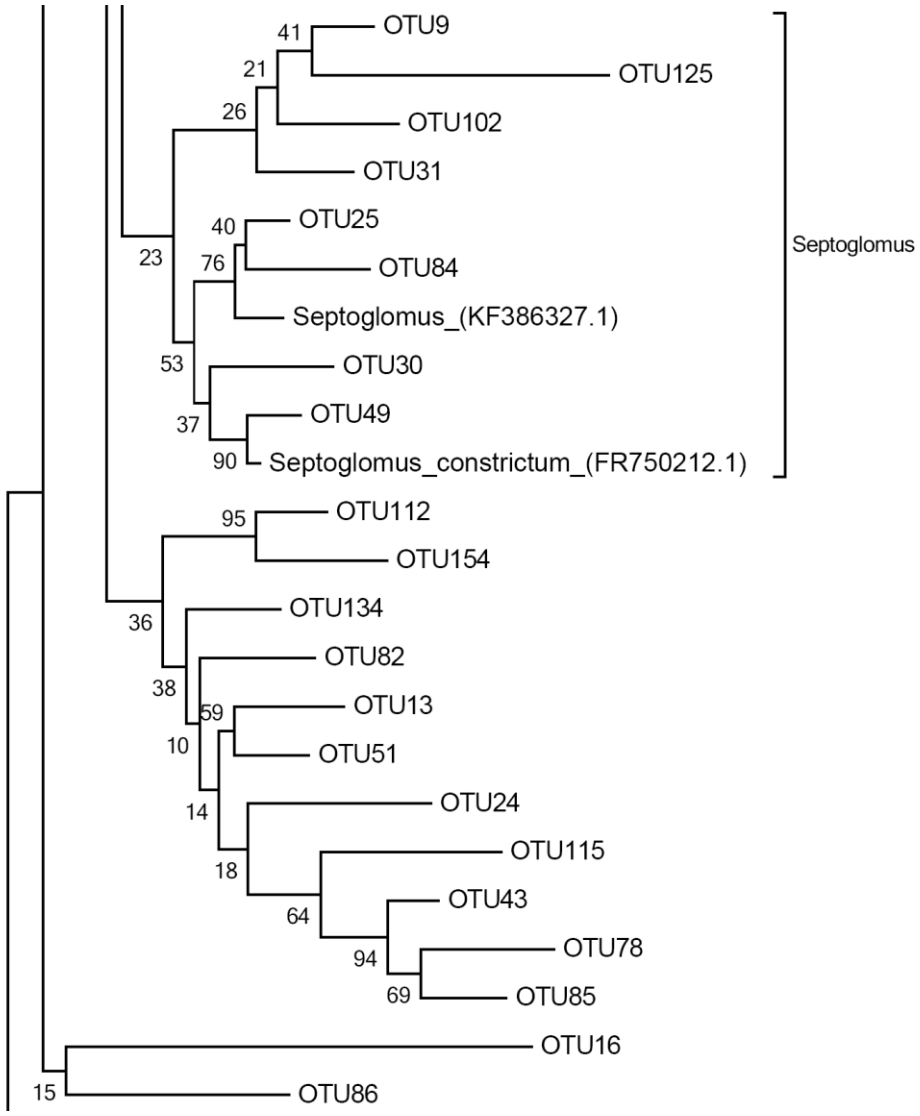
8.2. Appendix B: Effects of AMF, *Rhizobium* and phosphorus treatments and their interactions on phylogeny and relative occurrence frequency of mycorrhizal communities in roots of field pea and lentil in 2012 and 2013.

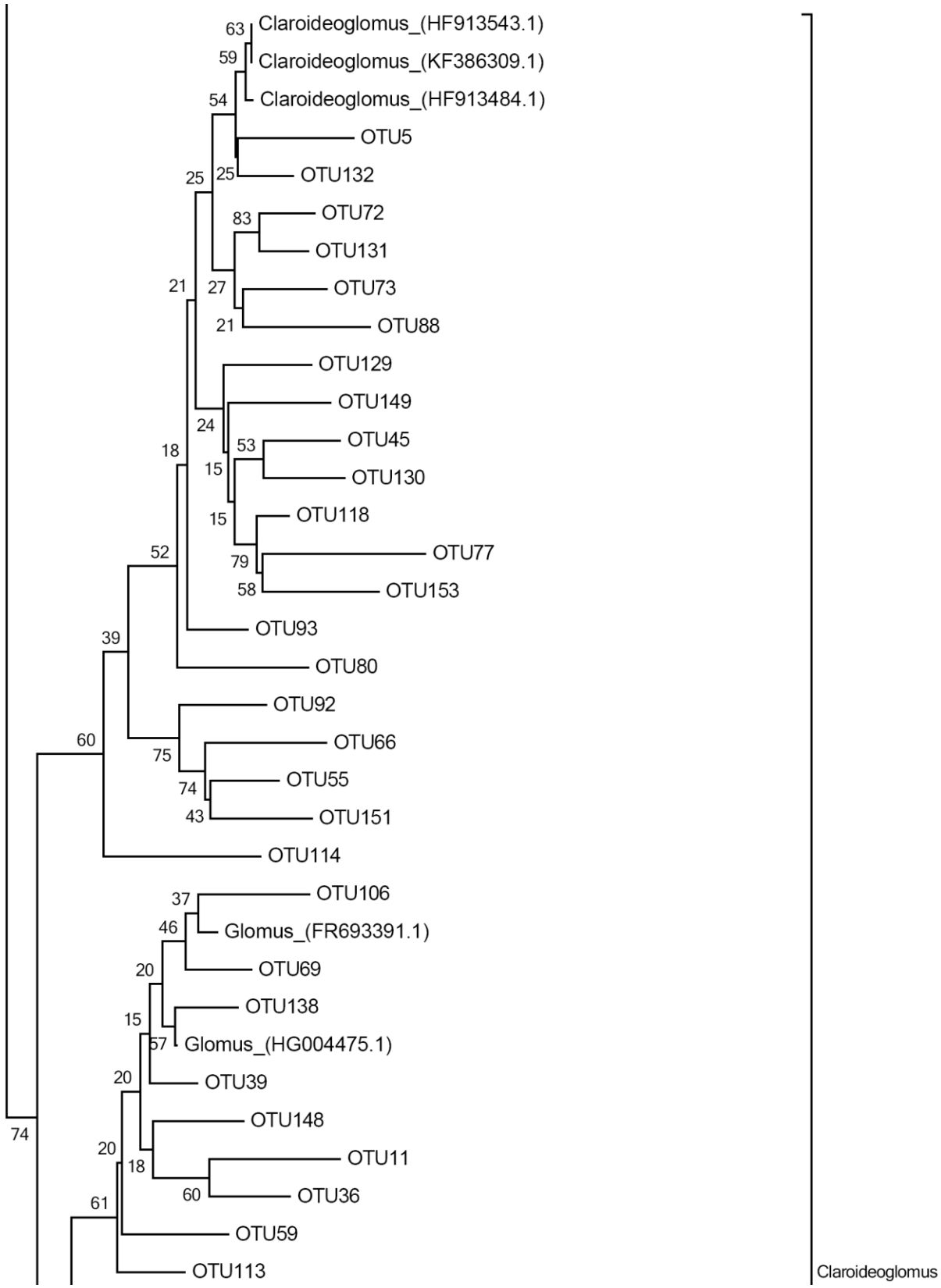



OTU27
OTU128
OTU38
Glomus_(JN644363.1)
OTU116
OTU143
OTU139
Rhizophagus_(HF559300.1)
Glomus_(JN644421.1)
Rhizophagus_(HG004448.1)
OTU141
OTU8
OTU37
Rhizophagus_(KF134509.1)
Glomus_(JX144122.1)
OTU44
Glomus_(AB899747.1)
AMF spore
Rhizophagus_irregularis_(HF968845.1)
Rhizophagus_irregularis_(HF968842.1)
Rhizophagus_irregularis(HF968835.1)
59
29
19
23
44
25
12
30
31
55
34
92
43
65
52
66
75
4
25
4
20
4


OTU57
OTU90
OTU12
OTU119
52
OTU2
22
OTU83
50
18
OTU100
4
OTU42
13
uncultured_Funneliformis_(HF913461.1)
17
Funneliformis_mosseae_(FR750227.1)
17
Funneliformis_mosseae_(FR750227.1)(2)
29
OTU111
16
0
OTU23
36
28
OTU46
Funneliformis
OTU103
OTU133
92
OTU63
57
Diversispora_aurantia_(AM713432.1)
27
OTU147
82
OTU33
52
51
OTU123
OTU41
61
OTU146
99
OTU75
34
44
Diversispora_(HF913521.1)
0
Diversispora_(HF913520.1)
32
OTU108
44
Diversispora_(HE615058.1)
99
OTU15
74
OTU74
Diversispora


OTU94
OTU95
OTU150
OTU87
OTU126
OTU4
Glomus_(JN644439.1)
OTU22
OTU58
Glomus_indicum_(GU059539.1)
OTU53
OTU56
OTU97
OTU109
OTU35
uncultured_Glomus_(FJ831571.1)
OTU152
OTU121
Glomus_(KC579423.1)
OTU140
OTU21
OTU135
OTU28
OTU6
OTU10
OTU89
OTU48
OTU47
OTU18
Glomus_(FR693482.1)
OTU122
OTU142
Glomus


OTU9
OTU125
OTU102
OTU31
OTU25
OTU84
Septoglomus_(KF386327.1)
OTU30
OTU49
Septoglomus_constrictum_(FR750212.1)
Septoglomus
OTU112
OTU154
OTU134
OTU82
OTU13
OTU51
OTU24
OTU115
OTU43
OTU78
OTU85
OTU16
OTU86
41
21
26
40
23
76
53
37
90
95
36
38
59
10
14
18
64
94
69
15


Claroideoglomus_(HF913543.1)
Claroideoglomus_(KF386309.1)
Claroideoglomus_(HF913484.1)
OTU5
OTU132
OTU72
OTU131
OTU73
OTU88
OTU129
OTU149
OTU45
OTU130
OTU118
OTU77
OTU153
OTU93
OTU80
OTU92
OTU66
OTU55
OTU151
OTU114
OTU106
Glomus_(FR693391.1)
OTU69
OTU138
Glomus_(HG004475.1)
OTU39
OTU148
OTU11
OTU36
OTU59
OTU113
Claroideoglomus

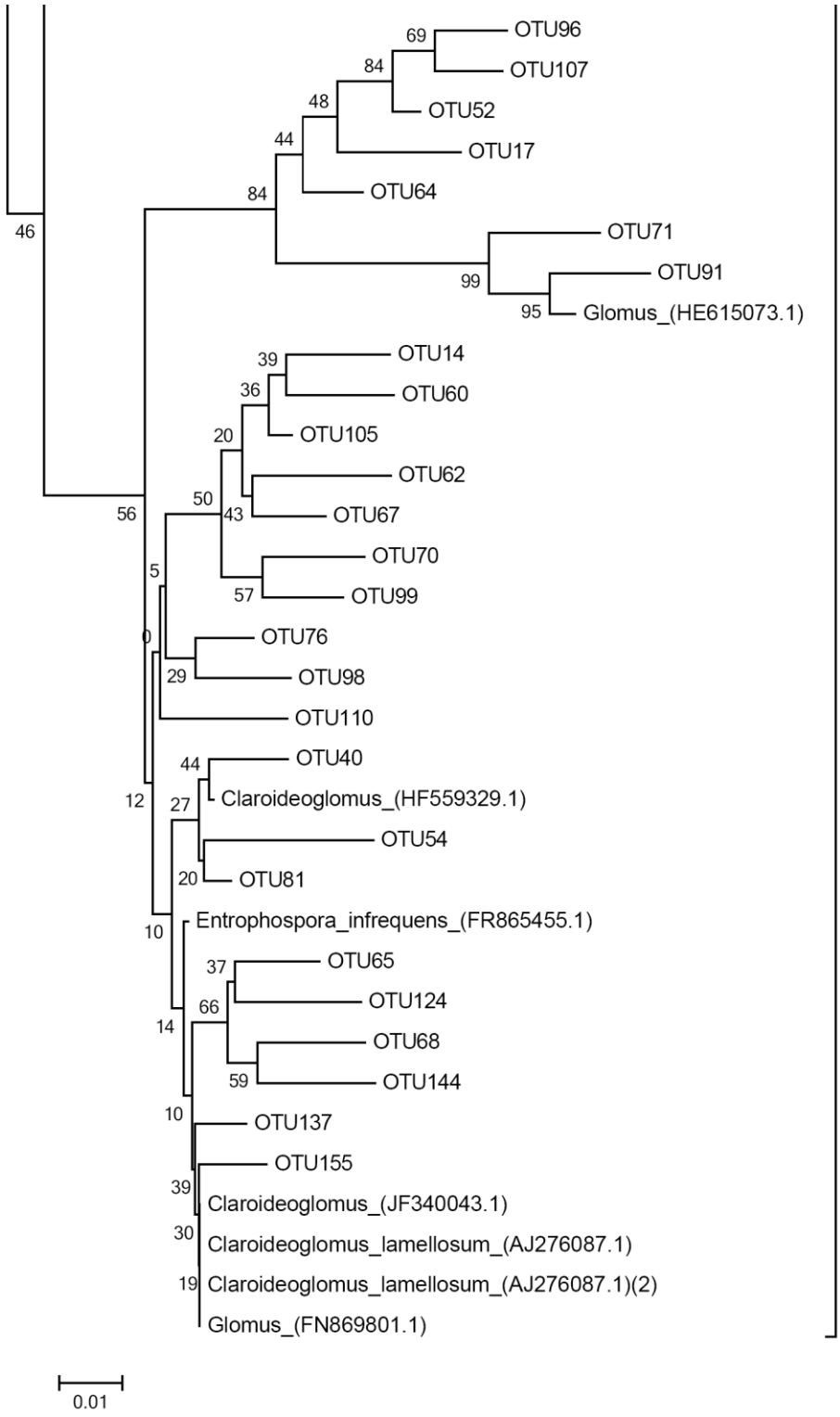


Fig. A. Phylogenetic tree showing phylogenetic position of different AMF OTUs identified from the entire experiment using BLAST. Bootstrap values above the branches are obtained from neighbour-joining analysis (bootstrap value 1000); these are shown only when >50% in at least one of the analyses.

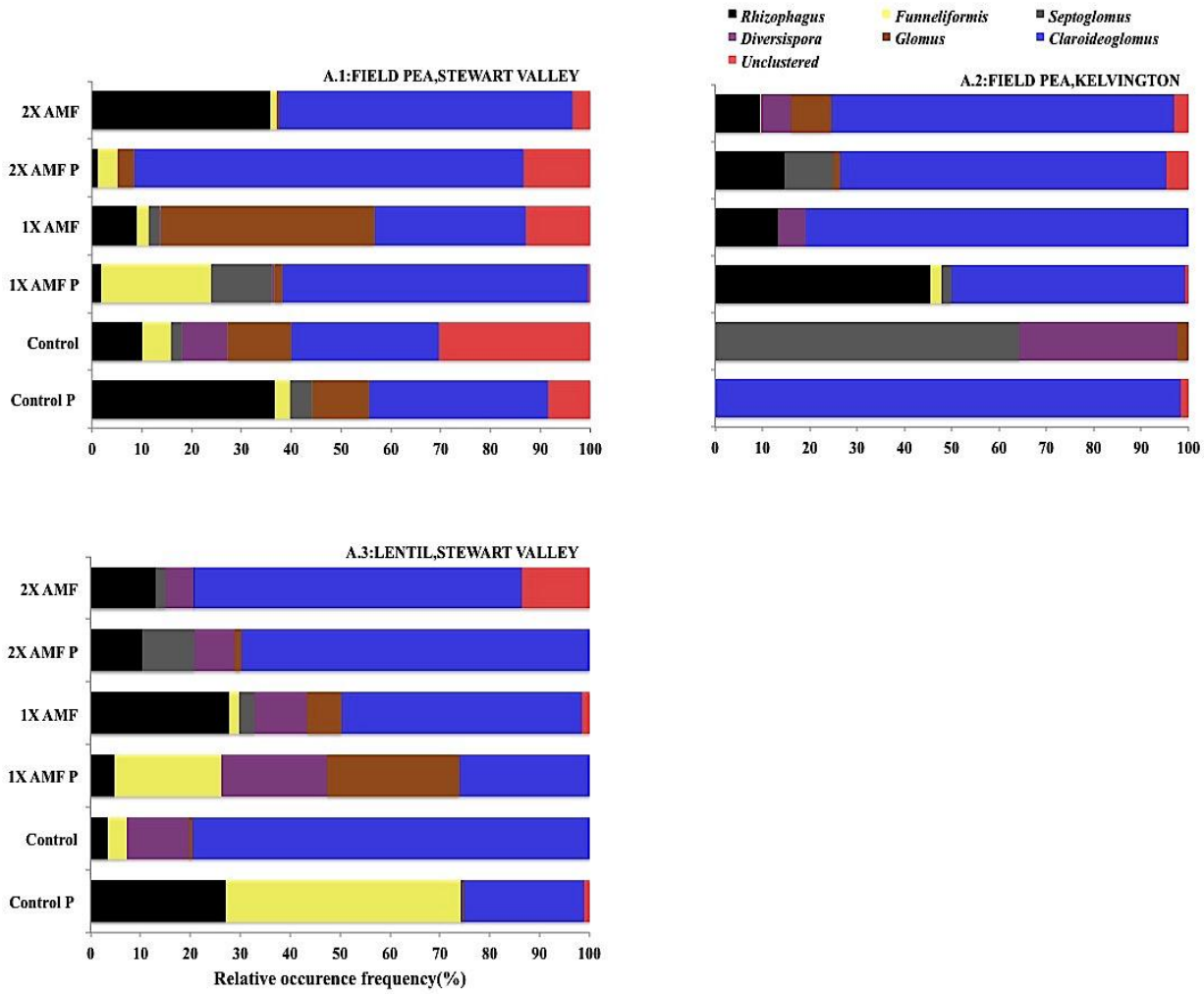


Fig B.1. Relative occurrence frequency of mycorrhizal community in roots in field pea and lentil in 2012. Three levels of arbuscular mycorrhizal fungi (AMF) treatment, 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.

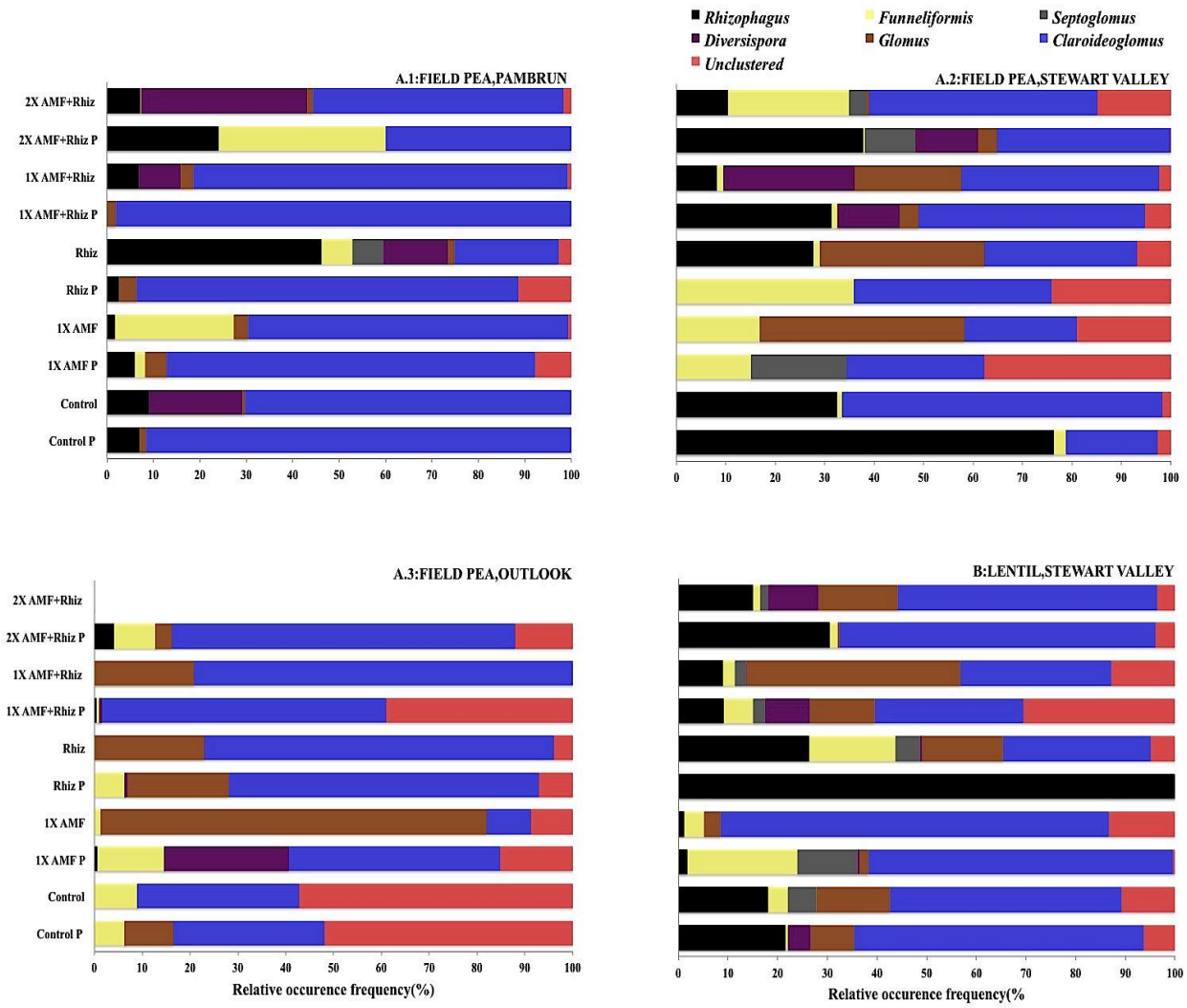


Fig B.2. Relative occurrence frequency of mycorrhizal community in roots in field pea and lentil in 2013. Three levels of arbuscular mycorrhizal fungi (AMF) treatment, 0 kg ha^{-1}, 7.5 kg ha^{-1} and 15 kg ha^{-1} are designated as 0X, 1X and 2X respectively. Two levels of P application, 0 and 16.8 kg ha^{-1} P_2O_5 as monoammonium phosphate.